A Summary of Current Program 7/1/65

and Preliminary Report of Progress

for 7/1/64 to 6/30/65

NORTHERN

UTILIZATION RESEARCH AND DEVELOPMENT

DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1964, and June 30, 1965. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

UNITED STATES DEPARTMENT OF AGRICULTURE

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TABLE OF CONTENTS

INTRODUCTION

The Northern Utilization Research and Development Division, located at Peoria, Illinois, is one of four research divisions of the Agricultural Research Service concerned with the development of basic knowledge of chemical composition and physical properties of farm commodities and with the application of this knowledge to the development of new or improved products and processing technology that will enhance utilization of these commodities. The other Utilization Research and Development Divisions are the Eastern at Philadelphia, Pennsylvania, the Southern at New Orleans, Louisiana, and the Western at Albany, California.

The need and importance of utilization research on farm commodities arise from the fact that the farmer is not organized to carry on modern scientific research to maintain old, and create new, markets for his products. The Northern Division is responsible for utilization research concerned with industrial utilization of cereal grains, soybeans and flaxseed and with food and feed uses of corn, sorghum and soybeans. Responsibility for food and feed uses of wheat is assigned to the Western Division. In the Department's program of research on replacement crops the Northern Division conducts all screening and characterization studies on uncultivated plants and their components and is responsible for more intensive utilization research on new oilseeds containing erucic acid and on new gum and pulp fiber plants. Research on additional selected new oilseeds is reported by the other three utilization research divisions. During the reporting period, the Northern Division maintained small programs on sugarcane and forages. The major part of the Department's utilization research on sugarcane and forages is reported by the Southern and Western Divisions, respectively.

In this report, utilization research of the Northern Division is discussed under the 11 Area Headings shown in the Table of Contents. For each area, a description of the current research program is provided, including domestic research contracts and grants and sponsorship of related research performed abroad under grants of Public Law 480 funds. A preliminary report of progress and a list of publications is given for each area for the period July 1, 1964, through June 30, 1965. A description of related work of State Experiment Stations is also provided for each area.

The scientific research effort at the Northern Division amounts to approximately 235 professional man-years. In addition, the Division supervises domestic research contracts equivalent to 45.7 professional man-years and grants equivalent to 12.1 professional man-years, and sponsors a comprehensive program of research comprising 52 PL 480 grants. Following are some of the recent utilization research accomplishments of the Northern Division.

Process for reducing the microbial content of flour. Department scientists have developed methods for treating wheats, as well as flours milled from them, to reduce the microbial population in the finished flours to very low levels--1 percent or less of the number present when the treatments are not used. The microorganism content of flours is important to the storage life of refrigerated, frozen, and cooked convenience foods. Therefore, specifications for the maximum permissible levels of bacteria, molds, and yeasts in flours are being included by most purchasers of flour for use in these convenience foods.

Without special treatments, flours are normally too high in microbial count for use in the convenience foods, despite the fact that 90-95 percent of the microorganism content of the wheat goes into the millfeed fraction. Our studies have shown that finished flours can be improved substantially by grain cleaning and the use of certain chemical agents in washing and tempering waters. However, when the level of microorganisms is high in the wheat, such treatments do not give a low enough count in the flour for critical uses. This difficulty has been overcome by developing processes based on using moderate heat treatment of the wheat before milling, or of the flour after milling. The finished flours have sufficiently low microbial count to meet specifications, yet show little or no heat damage to the flour.

Processes involving these heat treatments could be adapted to any mill with but moderate capital expenditures and with low operating costs.

Production of aflatoxin for research use. Aflatoxin is produced by certain fungi, especially Aspergillus flavus, and was discovered because it caused deaths of thousands of turkeys in England. Quantities of aflatoxin are needed for feeding large animals to determine its toxicity and its fate in animal tissues used for food and in dairy products and eggs. A successful and unique method developed involves the inoculation of sterile, moistened, polished rice in glass flasks with spores of Aspergillus flavus, which is then shaken as a solid substrate on a reciprocal shaker. Aflatoxin reaches levels of 1.2 grams per kilogram of substrate in 5 or 6 days. This yield is 10 times more aflatoxin than obtained in liquid media used by other laboratories. Consistent production of aflatoxin at high levels is attained. It can be extracted from the rice with ease. Fifteen grams of pure aflatoxin B_1 have been supplied for swine feeding, 4 grams of impure material sent to FDA for their studies, and a large number of reference standards have been prepared for use by researchers.

Polysaccharide gum from corn sugar commercialized. Four industrial companies are now producing "B-1459" water-soluble gum by the Department's process for bacterial fermentation of corn sugar. Solutions of the gum in water exhibit unusually high viscosities that are stable over wide ranges of temperature and concentrations of salt, acids and alkali. In contrast, presently available gums are deficient in one or more of these qualities.

Since Department feeding tests have shown negative toxicity, two of the companies have submitted requests for Food and Drug approval. The Department has assisted in commercialization of the gum by providing bacterial cultures and technical guidance and has cooperated with producers and possible users in evaluation of products and development of possible applications. The gum has a potential multimillion-pound market in industrial and food uses.

Standby process for reducing radioactivity in wheat. The Department has demonstrated a feasible method for the reduction of the radionuclide, strontium-90, in wheat and its milled products. Radioactive fallout is primarily deposited by rains, substantially in water-soluble form. This activity deposits on the foliage and seed head of the plant and in the soil. Under present low levels of atmospheric radioactivity, about 80-90 percent of the strontium-90 in wheat grain results from surface deposition of fallout and only 10-20 percent from absorption through the roots. In case of nuclear attack, a much higher proportion of the total radioactivity would be on the surface. The reduction of activity must be accomplished by treatment of the whole wheat berry before it is milled to flour and feed products.

A washing procedure has been developed which can be integrated into the milling process. Effectiveness results from the use of warm, dilute phosphoric or citric acid solutions, which are food acids. The wheat is washed in the solution for a period of 3-4 hours, dried, tempered and milled. When a hard winter wheat blend containing 395 picocurries strontium-90/kilogram was treated by this process, a total of about 75 percent of the strontium-90 was removed from the wheat. This was reflected in the milled fractions, the decrease in radioactivity being 81 percent for the bran, 71 percent for shorts, 45 percent for clears, and 27 percent for patent flour.

These results indicate that wheats can be decontaminated sufficiently for food purposes in times of emergency when high-density fallout might occur. The additional strontium-90 would be present in the outer layers where it can be largely removed. Also, the treatment of heavily contaminated wheat before milling offers the possibility of providing feed fractions that are considerably improved with respect to strontium-90 content.

Superior cooking oil for world market. ARS scientists have developed new laboratory methods having promise for producing a flavor-stable soybean cooking oil and at the same time preserving its high nutritional value. Of further importance is achievement of the improved stability needed to withstand storage and transportation for foreign marketing. These methods are based on the discovery of new catalysts that show increased selectivity in promoting hydrogenation of linolenate, the unstable component of soybean oil. With the aid of an analog computer, it has been demonstrated that linolenate content can be reduced to adequately low levels without the need for winterization to remove excessively hardened oil. Studies are in

progress on adaptation of these new methods for use by industry. Success should expedite the role of soybeans in supplying a large share of the annual world deficit of 4 billion pounds of food fats and oils.

Improving stability of linseed oil emulsion paints. ARS scientists have made an important contribution to expanding use of linseed emulsion paints by discovering a novel way to stabilize them. Linseed oil paints should contain zinc oxide to prevent growth of mildew on the paint film. In water-based paint systems, however, zinc oxide and titanium oxide, a white pigment used in most exterior paints, develop opposite electrical charges. The pigment particles attract each other and gradually clump together. This greatly shortens the storage life of the paint. Our scientists found that addition of a commercially available phosphate chemical overcame this problem and gave the paints the needed increase in storage life. This research also makes it possible to predict the occurrence of interactions between particles of other paint pigments that might be used, so that undesirable combinations can be avoided. Based on this and other pioneering ARS research, industrial production of linseed emulsion paints is making rapid gains. Three major producers are now marketing linseed emulsions for use in formulating paint. One of these alone is supplying emulsions to some 150 paint manufacturers. One of the nation's largest retailers is among those distributing linseed emulsion paints. The potential annual market exceeds 140 million pounds of linseed oil.

Crambe oil for the steel industry. Oil from crambe, a new crop being developed by the Department, has been evaluated in cooperation with industry as a mold lubricant for the continuous casting of steel. The results showed that crambe oil is superior to any other material for this purpose. In continuous casting, molten steel is poured through an oscillating mold. Proper lubrication of the mold is essential to ensure continuous emergence of a high-quality ingot. Continuous casting is a relatively new but rapidly growing process. Six years ago there were less than 20 installations throughout the world. Today there are over 100. The potential market for crambe oil in this process is 7 to 8 million pounds annually. Substantially increased plantings of crambe are planned this year to provide oil for this outlet and to encourage development of still further end uses.

Kenaf, a new potential raw material for papermaking. Department scientists have demonstrated that kenaf pulp can be used to make a wide range of types of paper having commercially acceptable properties. Kenaf, which is being studied in the Department's program on new crops, is an annual crop that can be grown in many areas of the U. S. Yields of over 10 tons per acre of dry matter have been readily obtained. Even yields as low as 6 tons per acre should make the growing of kenaf economically competitive with corn and wheat according to studies by Department scientists. As a result of these studies, industrial interest in kenaf as a pulp crop is increasing. At least five major paper companies are investigating kenaf through plantings and pulping studies.

AREA NO. 1: CEREAL STARCHES
INDUSTRIAL UTILIZATION

Problem. Starch accounts for about two-thirds the weight of all grains. Finding new, large-volume outlets for starch would, therefore, result in substantially increased consumption of cereal grains. Of the 5.6 billion pounds of cereal starch now produced, about 3.5 billion pounds is used ultimately in food products, and increases would be expected to follow population growth. However, the remaining 2.1 billion pounds find industrial outlets that offer opportunities for increases at a rate greater than that of population growth. Because starch must compete with products derived from nonagricultural sources, these opportunities can best be realized by a program of research designed both to maintain the competitive position of starch in its current uses and to develop economical new industrial uses.

Substantial new outlets for cereal starches and flours can be envisioned if basic research and development on several types of chemical and physical modification of starch and flour now in the experimental stage or anticipated can be prosecuted to successful conclusion. This research is mainly directed toward new products for the pulp and paper industries and for the building and construction industries, but other industries, such as the chemical, petroleum, mining, textile, plastics, coatings, and packaging industries, also provide attractive opportunities. New outlets for starch that appear very promising include use of modified starches as wet-strength additives for paper, water-resistant adhesives, coatings, and foamed products, and of starch-derived pulps as an integral part of high-quality paper. In addition, if the competitive position of starch is successfully maintained through improvement by research, additional consumption would be expected by 1975 from participation in the multimillion-bushel markets for grain resulting from normal growth of existing industrial outlets for starch and flour such as paper, textiles, packaging adhesives, drilling muds, and building materials.

To accelerate realization of these goals, more information is needed on the physical and chemical properties and chemical reactions of cereal starches, on economical methods for effecting desired physical and chemical modifications and on product evaluation and development. In addition, still further new markets for cereal starches should be possible from an adequate program of fundamental and exploratory research to discover new concepts, principles, and reactions leading to new processes and products for future development.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic and physical chemists and chemical engineers engaged

in basic, applied and developmental studies on the chemistry of cereal starches and their conversion to useful industrial products.

The Federal scientific effort for research on cereal starches totals 62.3 professional man-years. Of this number 19.1 are devoted to chemical composition and physical properties and 43.2 to new starch chemical derivatives and their evaluation.

Research at Peoria, Illinois, on chemical composition and physical properties (13.5 professional man-years) involves fundamental research on reactions of maltose and glucose, on amino acid and peptide derivatives of carbohydrates, and principles of graft polymerization. Research contracts (2.1 professional man-years) are in effect with the Arizona Agricultural Experiment Station, University of Arizona, Tucson, Arizona, for basic studies on the reaction of starch with mercaptans (.6 professional man-year) and on the reaction of acetylene with methyl glucoside (.6 professional man-year); with The Johns Hopkins University, Baltimore, Maryland, for basic research on the reactions of starch in fluid dynamic media (.7 professional man-year); and with Southern Illinois University, Carbondale, Illinois, for investigations on synthesis of maltooligosaccharides (.2 professional man-year). Contract research on the reaction of starch with acetylene was completed at the Arizona AES, University of Arizona, Tucson, Arizona. Grants (3.5 professional man-years) have been made to Ohio State University Research Foundation, Columbus, Ohio, for basic research on the reaction of vinyl ethers with carbohydrates (1.4 professional man-years); to Ohio State University, Columbus, Ohio, for basic investigations of unsaturated and sulfur-containing carbohydrates (.8 professional man-year); and to Purdue Research Foundation, Lafayette, Indiana, for studies on sugars containing carbon-bound nitrogen, phosphorus and sulfur (1.3 professional man-years).

Research at Peoria, Illinois, on new starch chemical derivatives and their evaluation (28.6 professional man-years) involves basic and applied studies on various types of chemical products derived from starch and dextrin and in evaluation of these products for various industrial uses such as pulp and paper products, plastics, coatings, organic chemicals and stable viscosity agents. Research contracts (14.6 professional man-years) are in effect with Ohio State University, Columbus, Ohio, for research on synthesis of amino derivatives of starch (1.2 professional man-years); with Battelle Memorial Institute, Columbus, Ohio, for developmental research on starch and other cereal grain xanthides (6.2 professional man-years) and for studies on starch derivatives for use as colloids in water-emulsion paints (1.2 professional man-years); with Stanford Research Institute, Menlo Park, California, for research on graft copolymers of cereal starches with vinyl-type monomers (1.3 professional man-years) and on process development of selected graft copolymers (2.3 professional man-years); with Archer Daniels Midland Company, Minneapolis, Minnesota, for evaluation of starch polyol urethane foams (2.0 professional man-years); and with Western Michigan University, Kalamazoo, Michigan, for evaluation of modified cyanoethylated starches for applications in paper (.4 professional man-year). During the reporting period, research

at the University of Minnesota, St. Paul, Minnesota, on reactions of dialdehyde starch in solution was completed.

The Department also sponsors research on cereal starches conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to National Institute of Agronomic Research, Paris, France, for research on changes induced in starch by gamma irradiation (4 years, 1961-1965); "Giuliana Ronzoni" Scientific Institute for Chemistry and Biochemistry, Milan, Italy, for research on glucopyranose rings in starches and dextrins (5 years, 1962-1967); Institute for Fibres and Forest Products, Jerusalem, Israel, for studies on the mechanism and products of mild oxidation of starch (5 years, 1963-1968); University of London, London, England, for research on debranching enzymes and their use in studying the fine structure of starch components (5 years, 1963-1968); University of Osaka Prefecture, Osaka, Japan, for development of an analytical method for carbonyl groups in carbohydrates (4 years, 1964-1968); and University of Edinburgh, Edinburgh, Scotland, for studies on the mechanism and structural changes involved in thermal, acid and alkaline degradation of starches (5 years, 1964-1969). During the reporting year research was completed on starch structure as revealed by interaction of starch and enzymes at the University of Birmingham, Birmingham, England, and on the proteolysis inhibiting effects of cereal starches and flours at the National Institute of Hygiene, Paris, France.

Research on new starch chemical derivatives and their evaluation involves grants to Hebrew University, Jerusalem, Israel, for studies on starch vinyl and epoxide graft copolymers (4 years, 1963-1967); Institute of Industrial Chemistry, Bologna, Italy, for studies on fatty chemical derivatives of starch dextrins (5 years, 1960-1965); National Institute of Technology, Rio de Janeiro, Brazil, for research on phosphorus- and sulfur-containing cationic starches (5 years, 1962-1967); Ahmedabad Textile Industry's Research Association, Ahmedabad, India, for research on starch-gum copolymers prepared by codextrinization (5 years, 1963-1968), and for studies on preparation and characterization of hydroxyethyl ethers of cereal starches (5 years, 1965-1970); Academy of Sciences and Chemical Institute "Boris Kidric," Ljubljana, Yugoslavia, for studies on modification of starch by moisture and temperature treatments (5 years, 1964-1969); and Plastics Research Institute TNO, Delft, The Netherlands, for research on preparation of metal alkoxides of starch for use as intermediates in synthesis (5 years, 1964-1969).

PROGRAM OF STATE EXPERIMENT STATIONS

State stations conduct a continuing program of research on the fundamental chemistry of cereal starches and their utilization. One regional project, NC-60, is directed to modification of starch for industrial uses. Participating States are seeking to: determine the fundamental reactions in the nonenzymatic dextrinization of starch; investigate chemical polymerization of D-glucose derivatives for the production of new types of synthetic

polymers; determine the mode of action of oxidants on starch; modify the basic structure of the D-glucose units in starch; discover enzymatic reactions which can modify starch and the effect of structural characteristics of starch on the action of enzymes; and develop methods by which nitrogen can be chemically attached to starch.

Other basic research is directed to study of the fundamental structure of complex carbohydrates and the mechanism of their formation and breakdown. Enzyme systems from plants and microorganisms are being examined from the viewpoint of their effect and role in structural changes, biosynthesis and deposition of starch. Systems of pure carbohydrases capable of attacking the whole spectrum of polysaccharide structure are being sought and their nature, mechanism and specific actions are being determined. Improved methods for separating and purifying a number of dextrin fractions continue to be studied. A new method for enzymatic synthesis of amylodextrins is being pursued.

Production of new types of hydrophilic polymers offers additional research challenges. Introduction of mercapto and amino groups as well as anhydro bridges in place of hydroxyl substituents is leading to additional families of modified polysaccharides. The resulting polymers are being characterized physically and biochemically.

Advances in carbohydrate chemistry continue to yield new and improved synthetic methods for preparing derivatives of both mono- and complex polysaccharides such as sulfated polysaccharides, heterocyclic sulfur sugars, new preparations of gentiobiose, more convenient introduction of isopropylidene groups, and novel syntheses of phosphorylated sugar acids. Studies of the reactivity and micro-structure of starch granules illustrates still another facet to the starch utilization program of the stations.

The total scientific effort of the State experiment stations is about 7.1 professional man-years devoted to this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Reactions of maltose and glucose. Deacetylation of 1-aminoglucose pentaacetate and 1-aminomaltose octaacetate with methanolic ammonia under identical conditions gave the N-acetyl derivatives of 1-aminoglucose and 1-aminomaltose, respectively. The latter retained 1 mole of acetamide as a crystalline addition compound that could be decomposed by boiling absolute ethanol but not by boiling ethyl acetate. Urea and 2-oxazolidone were shown to form 1:1 addition compounds with D-glucose and maltose that can be used to direct the course of chemical reactions of these sugars to form new derivatives. Thus, acetylation and alcoholysis proceed differently with the complexes than with the free sugars. The complexing amide does not react to combine with the products. Further instrumental examination confirmed that

alcoholated hydroxide ion is the absorbing species involved in near-infrared determination of hydroxide ion in aqueous and nonaqueous alcohol. Other studies showed that the enhanced optical rotation of β-methyl glucoside in alcoholic sodium and other alkali-metal hydroxides is not due to alkoxide formation but more probably is caused by distortion in the cyclic conformation resulting from adduct and chelate formation.

A method for preparing mixtures of C-4 methylated maltooligosaccharides was worked out at Southern Illinois University. Also, a series of 4-O-methyl maltooligosaccharides of degree of polymerization 2 to 7 has been prepared.

2. Reactions of carbohydrates with acetylene and mercaptans. At the University of Arizona, research on vinylation of starch has been completed. In the final phase of the work attempts to polymerize vinyl starch with a cationic catalyst indicated crosslinkage through acetal groups rather than through polymerization of the vinyl ether groups. Films crosslinked after being cast were of poor quality. Initial studies at Arizona on the related vinylation of methyl glucoside were largely devoted to development of needed separation and analytical procedures and to synthesis of needed reference compounds. A new monovinyl compound has been isolated and characterized as methyl 3-O-vinyl-α-D-glucopyranoside.

In the research on polymeric mercaptals, also in progress at the University of Arizona, no reaction was observed between 1,10-decanedithiol and starch or, more unexpectedly, dialdehyde starch. Statistically designed experimentation provided useful information on the relationships among conditions, extent of reaction and properties of products in the reaction between glucose and dimercaptans.

3. Amino acid and peptide derivatives of starch. Treatment of methyl α-D-glucoside with sulfur monochloride selectively produced the 6-chloro-6-deoxy derivative. Glucose and carbobenzoxy-glycine activated by N,N-dicyclohexylcarbodiimide yielded a resolvable mixture of two mono- and one di-(aminoacyl) glucose derivatives. Studies were conducted to obtain further basic information on the influence of structure in the use of S_2Cl_2 to replace hydroxyl groups with chlorine. This research on the synthesis of chloroglucose derivatives is directed to the possibility of alkylating protein nitrogen with a chloro-sugar. Successful preparation of specific aminoacyl sugars will enable needed determination of their properties.

4. New derivatives of starch and related carbohydrates. Under the grant at Ohio State University for research on unsaturated and sulfur-containing derivatives, two methods have been devised for introducing a double bond in place of cis- or trans-diol groups in glucose derivatives. This research has been strengthened by a grant to Purdue Research Foundation for research on new derivatives containing nitrogen, sulfur or phosphorus. In other studies at Ohio State, preliminary results of research on the reaction of vinyl ethers with carbohydrates indicate that no single hydroxyl group of a sugar reacts preferentially.

5. Basic studies on graft polymerization. Research on graft copolymerization comprised studies on the effects of reaction conditions, on fractionation of reaction products and on development of a method for enzymatic hydrolysis of the starch portion of the graft copolymer. Replication of the ceric-ion catalyzed grafting of acrylonitrile to gelatinized wheat starch showed that reproducibility was good. Molecular weight of the grafted chains ranged from 710,000 to 840,000 and graft site frequency was 3,940 to 4,600 anhydroglucose units per graft chain.

Partial hydrolysis of these starch-acrylonitrile graft copolymers is the obvious route to obtain water-dispersible products for industrial applications. The reproducibility of the graft copolymerization reactions indicates that final products having acceptably constant properties from batch to batch should be obtained without great difficulty.

6. Application of nuclear magnetic resonance to starch structure and chemistry. This newly undertaken research is designed to utilize nuclear magnetic resonance, a powerful new instrumental technique for characterization of organic molecules, in the solution of problems relating to the chemistry and structure of starch, starch components and their chemical derivatives. These studies already have provided good evidence for definite complex formation between OH groups and certain proton acceptors. Observations were made with the aid of an instrument which minimizes transient signals or "noise." The results strongly indicated the existence of the helical configuration for amylose in solution. Previously the only evidence for this configuration was derived from X-ray patterns of solid materials or deduced from chemical reactivity. Unlike NMR, older conventional procedures could not be used effectively for measurements on dilute solutions.

7. Reactions of starch in fluid dynamic media. Encouraging results have been obtained in initial trials at The Johns Hopkins University of the fluidized bed reactor for converting starch to levoglucosan (see previous annual report). Variation of the dielectric constant of starch with frequency was found to be too small to serve as the basis of a practical analytical method for starch.

8. Proteolysis inhibition by starch. Under a PL 480 grant at the National Institute of Hygiene, Paris, France, studies have been made on the physiological activity of concentrates of wheat antitryptic factor. Protein digestion in rats was inhibited by high levels of the factor. Laboratory work on this project has been completed. The final report, which has not yet been received, is expected to provide information on the nature of this effect on digestion and its possible relationship to nutrition.

9. Starch structure and degradation. At the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, scientists working under a PL 480 grant have completed phases of research involving infrared spectra of amylose and its oligomers and hydroxyl proton resonances of sugars

in dimethyl-sulfoxide solution. This research is part of detailed investigations on the glucopyranose ring structure of starch.

In studies under a PL 480 grant at the University of Birmingham, Birmingham, England, theoretical equations were derived to explain sedimentation and electrophoresis patterns of interacting polymers, the equations being applied specifically to β-lactoglobulin. A method was developed for obtaining synthetic amylose having a high molecular weight, no branches, and a narrow size distribution. The product may be a useful model for amylose. These studies have been completed, but the final report has not yet been received.

At the University of London, London, England, under a PL 480 grant for research on starch- and glycogen-debranching enzymes, a method has been devised for determination of the average chain length or degree of branching of amylopectin by its complete enzymatic hydrolysis with β-amylase and pullulanase, followed by measurement of the amount of glucose in the resulting mixture of maltose and glucose. A branching enzyme has been isolated from sweet corn endosperm and its mechanism of action has been determined.

Studies on thermal degradation of starch and its components have been initiated at the University of Edinburgh, Edinburgh, Scotland. Results so far indicate the absence of an induction period or autocatalysis in the decompositions.

10. Mild oxidation of starch. In research under a PL 480 grant to the Institute for Fibres and Forest Products, Jerusalem, Israel, study of the oxidation of starch by bromine in acid medium has revealed the existence of a bromine-starch complex. Another interesting discovery is that the iodine affinity of wheat starch is increased by disintegration of native aggregates in acid and neutral solution.

B. New Starch Chemical Derivatives and Their Evaluation

1. Chemical studies on starch xanthates and xanthides. Laboratory experiments showed that starch xanthate (D.S. 0.3) analyzed 10 minutes after discharge from the Ko-Kneader had 80 percent of the xanthate groups on secondary hydroxyls and 20 percent on primary hydroxyls. On aging for 3 days the xanthate groups approached equal distribution on the primary and secondary positions. When a highly viscous Ko-Kneader xanthate (from reactant sufficient to give D.S. 0.5 and 50 percent solids) was aged in sealed containers, the D.S. increased from an initial value of 0.29 to 0.43 in 4 hours and then decreased to near 0.29 in 6 days. Initial xanthate distribution favored the secondary position, but after a few hours 75 percent of the xanthate groups occupied primary positions.

Oxidation with lead tetraacetate was shown to be a useful tool for study of xanthides and other sulfur derivatives or carbohydrates. The structures $(RO)_2C=S$ and $(RS)_2C=S$ are oxidized by one mole of the tetraacetate to the

structures $(RO)_2C=O$ and $(RS)_2C=O$, respectively, whereas the -SH group is converted by one-half mole to -S-S-.

A series of experiments on the effect of reaction conditions on the oxidative coupling of starch xanthate using chlorine indicated that, except for minor compensable differences, chlorine and hypochlorite display equivalent effectiveness.

2. Process and product development research on starch xanthates and xanthides. Studies in cooperation with Forest Products Laboratory continue to indicate good potential for xanthide as a means of improving resistance of linerboard to high humidity. For corrugated board, starch xanthides (D.S. 0.07-0.25) produced significant improvement in flat crush and wet and dry tensile strength. On the other hand, in insulation board, use of cereal xanthates of D.S. 0.25 or less gave little if any improvement in wet strength.

Engineering research resulted in a process for xanthation of starch based on the use of starch pastes of sufficient fluidity to permit transport and mixing with sodium hydroxide solution and carbon disulfide by means of ordinary pumps and other conventional equipment. Xanthates prepared by this method were found to be equivalent in handsheet tests to those of the same D.S. prepared in the Ko-Kneader. Further investigation revealed that trisodium phosphate (TSP) was sufficiently alkaline to replace sodium hydroxide. Use of TSP enhanced ease of precipitation of xanthides from solutions of xanthates having a D.S. as low as 0.03. Other studies provided data needed for design of a fully continuous xanthation process based on these findings.

In studies to find optimum methods for incorporating xanthate into paper, a continuous process for oxidative crosslinking of starch xanthate in pulp furnishes has been devised and is being scaled up to handle 5 pounds of pulp per minute. In preliminary tests, the process resulted in exceptionally high effectiveness of xanthide in improving the properties of paper.

The xanthation phase of the contract project at Battelle Memorial Institute on production of xanthides with larger scale equipment has been completed. At throughput rates of 100 to 200 pounds of starch and CS_2-starch mole ratios of 0.1 to 0.3, xanthation efficiencies for samples aged 5 minutes are similar to those observed at the Northern Division for samples aged 10 minutes to 1 hour after discharge from the Ko-Kneader. Removal of sulfur byproducts from xanthate dispersions was achieved by continuous countercurrent contact of air with the xanthate acidified to pH 6.

Battelle xanthates were found to require several times the amount of hypochlorite for *in situ* crosslinking that was established for xanthates prepared at the Northern Division. Production of satisfactory handsheets also presented problems. These difficulties have been resolved with the discovery that differences in pH and hardness of water were responsible for the discrepancies.

Colloidal *ex situ* crosslinked xanthides were prepared at Battelle by two methods. These products had activity as wet-strength agents paralleling that of *in situ* crosslinked xanthide. Although in their present state of development the methods used would not be commercially practical, the successful preparation of *ex situ* crosslinked xanthides comparable to *in situ* products in activity as wet-strength agents is a very important result in view of the probable greater industrial attractiveness of an *ex situ* crosslinked product.

The results of the extensive research program summarized in this and the preceding section show that excellent progress has been made toward development of economical and industrially attractive processes for preparing cereal xanthates and for incorporating them into paper products having optimum properties.

3. Starch polyol foams. Statistically designed experiments revealed the optimum conditions for the acid-catalyzed glycolysis of starch. A method was found for avoiding premature gel formation in this step. Replicate experiments showed that the subsequent reaction with propylene oxide formed polyethers with highly reproducible hydroxyl number (413 ± 2) and viscosity (44,000 ± 1,500 c.p.s.). Major components of the crude glycoside mixture obtained by reacting starch and ethylene glycol were shown to be glycol α-D-glucoside (45-48 percent); glycol β-D-glucoside (21-24 percent), and glycol diglucosides (11 percent).

In the studies at Archer Daniels Midland Company on evaluation of plastic foams based on starch-derived glycol glucosides, laboratory-scale polyether preparations, foam screening tests and preliminary pilot-plant work were essentially completed. An improvement in the glucoside synthesis was devised that has the advantages of requiring less excess glycol and of avoiding gelation.

4. Graft copolymers. At Stanford Research Institute detailed studies showed that in grafting acrylic acid to pre-irradiated starch granules suspended in water, the reaction is complete in about 2 hours. A longer reaction time merely increased homopolymer formation. A study of solvents for graft copolymerization of starch and acrylamide showed that grafts with high acrylamide content were obtained with aqueous glycerol and sorbitol. The molecular weight of the grafted chains was higher with these two solvents than with the previously used aqueous ethylene glycol. High levels of add-on were obtained in the ceric-ion catalyzed graft copolymerization of starch with ethyl and butyl acrylates and methyl methacrylate, but not with methyl acrylate. A small pilot plant for continuous grafting of acrylonitrile to starch by ceric ion catalysts was designed and constructed. In the pilot-plant products, the grafts were shorter and more frequent than those of laboratory preparations. These changed characteristics in comparison to earlier laboratory preparations are highly desirable in terms of improved solubility, melt temperature and other important properties. A

100-pound sample produced in the continuous reactor was shipped to the Northern Division for evaluation.

In research under a PL 480 grant to the Hebrew University, Jerusalem, Israel, laboratory methods have been developed for the anionic graft polymerization of acrylonitrile, methacrylonitrile, methyl methacrylate, and propylene oxide onto starch and dextrin. The anionic method gives products with shorter and more frequent grafts than have been obtained thus far in other studies with free radical processes such as those described in the preceding paragraph. The anionically prepared products will therefore be most useful in relating graft size and frequency to properties. However, solvents other than dimethyl sulfoxide and tetrahydrofuran, those currently used in anionic grafting, will be required for a practical process.

5. Dialdehyde starch-protein plywood glues. The technique of preparing soyflour-blood-dialdehyde starch glue has been simplified and an improved formulation at lower cost has been achieved. In cooperative tests at Forest Products Laboratory, southern pine plywood bonded with this glue was essentially equivalent to pine plywood bonded with a commercial blood-phenolic resin glue. The experimental plywood has also shown good durability in 6 months of outdoor exposure. These results encourage the expectation that this new plywood glue will achieve commercial use.

6. New chemical products from starch and dextrin. The reaction of starch xanthate in water with amino compounds such as ethylene diamine, decamethylene diamine, and polyethylenimine yielded gels whose rigidity was found to depend on xanthate D.S., xanthate-amine ratio, amine molecular weight, and amount of water. Some of the gels displayed interesting adhesive and elastic properties that justify exploration of their potential as wet-end additives to paper as adhesives and sealants.

Starch xanthide and zinc starch xanthate have been compounded with natural and synthetic elastomers. The starch derivatives were compatible with the polymers even at the equal-weight level. Tensile strengths of the elastomers were increased several hundred percent by incorporation of 30-80 parts of cereal derivative per hundred parts of elastomer. Elongation and swelling in benzene decreased as starch content increased. There was little loss of starch derivative on extraction with hot water. In some experiments the starch derivative served as accelerator, curing agent and filler. Products varied from highly elastic materials to compositions resembling vinyl floor tile. Both of these discoveries--gel formation and compounding with elastomers--appear very promising and could lead to new outlets for cereal products.

A series of products was prepared by cyanoethylating cationic (aminoalkylated) starches. Cyanoethylation did not improve the properties of the cationic starches for various applications in paper. Detailed evaluation of low D.S. cyanoethylated starches for applications in paper manufacture are being undertaken in contract research by Western Michigan University. Contract

research to investigate starch derivatives for use in water emulsion paints will be performed by Battelle Memorial Institute.

In contract research on amino sugars at Ohio State University, derivatives of 3,6-diamino-3,6-dideoxy-D-altrose and of 2,6-diamino-2,6-dideoxy-D-mannose have been synthesized. Progress is being made in efforts to prepare 2-amino-2-deoxyamylose and 3-amino-3-deoxyamylose.

Under a PL 480 grant to the Institute of Industrial Chemistry, Bologna, Italy, significant progress was made in preparing, characterizing, and testing amino dextrins and fatty acyl dextrin derivatives. The amino dextrins, containing 0.1 to 0.5 percent nitrogen, are new products with adhesive properties which show potential uses as emulsifying agents in agricultural pesticide formulations and possibly also in printing inks and paper sizings. Also under a PL 480 grant, scientists at the Ahmedabad Textile Industry's Research Association, Ahmedabad, India, have carried out heat degradation of corn starch and gum karaya separately and in admixture in the temperature range 100-200° C. Evaluation at the Northern Division showed that a product from a mixture having a 9:1 ratio of starch to gum dextrin possessed some deflocculant ability.

Several cationic starch derivatives containing phosphorus and sulfur moieties have been prepared under a PL 480 grant at the National Institute of Technology, Rio de Janeiro, Brazil. Evaluation of the products at the Northern Division for cationic properties and for their fire-retarding properties in urethane foams gave disappointing results. Other research under PL 480 grants involves studies on preparation of metal alkoxides of starch at the Plastics Research Institute, TNO, Delft, The Netherlands; on hydroxyethylation of starch at the Ahmedabad Textile Industry's Research Association, Ahmedabad, India; and on modification of starch granules to obtain new paste properties at the Academy of Sciences and Chemical Institute "Boris Kidric," Ljubljana, Yugoslavia. No important results under these projects were reported during the past year.

7. Evaluation of starch derivatives in paper and paper products. Research on new chemical products from starch is supported by evaluation studies to determine the quality and performance of these products in applications in the pulp, paper and paperboard industry. During the reporting period tests were conducted, for example, with starch xanthates and xanthides and hetero-substituted starches. Results on the evaluation studies are reported in conjunction with the general discussion of research on the specific starch product. As a part of this work, variables involved in the crosslinking and handsheet procedures used in evaluation of starch xanthates and xanthides have been identified and can now be adequately controlled. The resulting improved reproducibility has resulted in more effective screening operations.

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AREA NO. 2: WHEAT
INDUSTRIAL UTILIZATION

Problem. Wheat traditionally commands a higher price than corn. Since the starch content and starch properties of these two cereals are similar, new industrial uses for wheat must rely on advantages to be obtained from other components. Wheat flour is a mixture of starch, protein, gums, fiber, and fat. Because of the simultaneous presence of these basic ingredients, opportunities are promising for development of a wide variety of industrial products from wheat flour that would be expected to have properties and uses different from those of related products derived from refined starch. The problem is to find means for economical modification and reaction of these ingredients with each other and with other chemicals in order to realize the potential of the combinations.

Basic research now being conducted by the Department points to new potential industrial uses for cereal starches and flours that could consume significant quantities of grain by 1975. Among potential outlets for wheat flour are sizes for many special grades of paper, cereal pulps that would form an integral part of such papers, and plastic or foamed compositions for hardboard and insulating boards. The opportunity for successful realization of these possibilities is enhanced by recently developed fine-grinding and air-classification milling techniques that permit the composition of flour to be varied over wide ranges. These techniques are now satisfactory for soft wheats, but ways must be found to adapt them to hard wheats which constitute over 90 percent of the wheat remaining after current needs have been met.

Wheat flour could achieve its share of potential new markets more rapidly, and discovery of additional new uses under both public and private research programs would be facilitated, if more information were available on the basic physical properties and chemical reactions of flour and its components, on tempering and milling techniques, and on processing methods for economical conversion of flour to desired end products.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing long-range program of research involving analytical, organic and physical chemists, chemical engineers and structural biologists engaged in basic studies of the chemical and physical properties of wheat, flour, flour fractions, and protein components and in applied research leading to new and improved wheat products for industrial use.

The Federal scientific effort for research on industrial utilization of wheat totals 44.8 professional man-years. Of this number 16.9 are devoted to chemical composition and physical properties, 12.7 to industrial chemical products, and 15.2 to processing technology.

Research at Peoria, Illinois, on chemical composition and physical properties (12.6 professional man-years) includes separation, characterization and chemical reactions of the component proteins of wheat gluten. Research contracts (2.4 professional man-years) are in effect at Purdue University, Lafayette, Indiana, for fundamental studies of the alkaline desulfurization of gluten (.8 professional man-year); and IIT Research Institute, Chicago, Illinois, for investigation of chemical modification of polypeptides obtained by hydrolysis of gluten (1.6 professional man-years). During the reporting period studies on methods for controlled hydrolysis of gluten, conducted at IIT Research Institute, Chicago, Illinois, were completed. Grants (1.9 professional man-years) have been made to Marquette University, Milwaukee, Wisconsin, for studies on synthesis and hydrolysis of amino acid glycosides (.5 professional man-year); to Kansas State University, Manhattan, Kansas, for investigations on separation of enzymes and proteins by disc electrophoresis (.8 professional man-year); and to Purdue Research Foundation, Lafayette, Indiana, for research on the effects of disulfide bond cleavage on the structure of wheat and corn endosperm proteins (.6 professional man-year).

Investigations on industrial chemical products conducted at Peoria, Illinois, (7.1 professional man-years) involve preparation and evaluation of new types of water-soluble and water-insoluble flour derivatives for industrial use. During the reporting period research specifically directed to wheat flour xanthates and xanthides was terminated, and engineering studies on acid-modified flour were completed. Further studies on wheat-derived xanthates and xanthides are being continued under contract research (see below) and under in-house projects broadly applicable to starch and flour xanthates and xanthides (see Area No. 1). Research contracts (5.6 professional man-years) are in effect with Iowa State University, Ames, Iowa, for engineering studies on use of pneumatic fluidization to effect acid modification of flour (.9 professional man-year); with Battelle Memorial Institute, Columbus, Ohio, for studies on preparation of xanthates from wheat bran and ground whole wheat and their use in making bag and box paper (3.0 professional man-years); and with Brown Paper Company, Berlin, New Hampshire, for evaluation of acid-modified flour as a surface-sizing agent for paper (1.7 professional man-years).

Processing technology research at Peoria, Illinois, (17.6 professional man-years) involves studies on conditioning and milling of wheat, air classification of flours, and reduction of viable microorganisms and radioactive contamination in wheat flour. A research contract (1.2 professional man-years) is in effect with the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on varietal variations in kernel properties and milling and fractionation characteristics of wheat. A grant has been made to Iowa State University, Ames, Iowa, for basic research on heat, mass and momentum transport (1.4 professional man-years).

The Department also sponsors research in this area conducted by foreign institutions under grants of PL 480 funds. Research on processing technology involves a grant to the Cereals Research Station, Research Association of British Flour-Millers, St. Albans, England, for investigations on quantitative measurement of properties of wheat that change significantly during conditioning (5 years, 1961-1966).

PROGRAM OF STATE EXPERIMENT STATIONS

Station research on use of wheat for purposes other than food has been limited. Some work is being devoted to economic feasibility studies related to use of wheat as a livestock feed when prices are competitive with prices of feed grains. Consideration is also being given to the supply and flow of wheats of different quality.

The total professional man-years devoted to industrial utilization of wheat is .6.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Characterization of wheat gluten proteins. Optical rotatory studies showed conclusively that gluten, glutenin and gliadin all contain α-helix. Glutenin and gliadin contained 17 and 25 percent α-helix, respectively. Gluten had 20 percent, which is near the expected average of the two fractions. Molecular weights of gluten and gliadin were determined with a membrane osmometer. Values for gluten varied from 50,000 to 220,000 and values for gliadin from 75,000 to 81,000, depending on conditions (i.e., use of urea or aluminum lactate buffer, varying ionic strength and pH). Lower molecular weights were obtained in the presence of urea than in its absence. In the presence of urea, high pH decreased the molecular weight of gluten but change in ionic strength did not. Changes in pH and ionic strength did not affect the molecular weight of gliadin in urea solutions.

Properties of reduced glutenin reoxidized at 5 percent concentration indicated that it had more intermolecular disulfide bonds and a more highly branched structure than native glutenin. The number of ionizable groups and their pK's were determined for whole gluten proteins. Indications were found that the conformation of these proteins depends on pH and is different in alkaline than in acid solution.

This research is being expanded through a grant to Kansas State University for research on disc electrophoresis as a method for large-scale separation of proteins and enzymes.

2. Chemical reactions of wheat gluten. Studies on reactivity of functional groups important to wheat gluten chemistry showed that in comparable polar and steric environments SH groups reacted with acrylonitrile 300 times faster

than did NH_2 groups. The nature of the vinyl compound also importantly affected reaction rate. Thus, methyl vinyl ketone reacted with glycine 700 times faster than did acrylamide. Whole gluten was completely derivatized by reaction with butyl acrylate. Only the lysine residues in the protein were involved in this reaction. Under optimum conditions for balance between hydrazide formation and peptide cleavage in hydrazinolysis of gluten, 26 percent of the primary amide groups were replaced by hydrazide residues and 10 percent of the peptide bonds were cleaved. These results show that progress is being made toward the goal--preparation of proteins modified in specific and pre-determined ways to adapt them to particular industrial end-uses.

Final results of research on gluten hydrolysis at IIT Research Institute indicated uniformity of charge distribution for the hydrolyzate. This observation, together with the previous demonstration of uniform particle size, indicates that polypeptides in the hydrolyzates have definite promise as raw materials for possible industrial products. Research to further characterize and to investigate chemical modification of these polypeptides will be conducted under a new contract.

Studies at Purdue University confirmed the greater rate of sulfur liberation from commercial gluten as compared to laboratory-prepared gluten. Kinetic studies were initiated on the release of sulfide sulfur, hydrolysis of amide groups and hydrolysis of peptide linkages. Significant differences were observed in the rate and extent of sulfide release between gliadin and glutenin when treated at different temperatures with different concentrations of alkali. This research is being strengthened by a grant to Purdue University providing for basic chemical studies on disulfide bond cleavage, a reaction of fundamental importance to the studies on desulfurization.

A grant has been made to Marquette University for studies on synthesis and degradation of O-glycosides of hydroxy amino acids that form protein to carbohydrate linkages in glycoproteins.

B. Industrial Chemical Products

1. Acid-modified flour. In the final phase of engineering research on acid-modified flour a pneumatic reactor was designed, operated and developed to a point permitting the flour to be heated to 140° F. with negligible loss of moisture. These studies are now complete. A detailed evaluation of acid-modified flour as a paper size will be carried out under a contract with Brown Paper Company.

2. Fluidization of flour. In contract research at Iowa State University agglomerated flour was observed to behave ideally in a fluidized bed. For ordinary flour, addition of small amounts of silica or calcium silicate was required to secure good fluidization. In other tests, the rate of adsorption of hydrogen chloride on flour was 3 g. per 100 g. of flour per minute under ordinary moisture and temperature conditions. Rates as high as 10 g. per

100 g. per minute have been noted under some conditions. The limiting factor appeared to be the amount of water already adsorbed by the flour. Hydrogen chloride was more strongly bound by flour than is water, and there was significant heat of adsorption. In addition to this work, further information was obtained pertinent to the final design of a cylindrical column device for fluidizing flour.

3. Chemical modification of flour. Studies were made of reagents such as alkylenimines and dialkyl aminoalkyl halides for dry-state preparation of cationic flours. Ethylenimine appeared very promising since flours treated at 98° C. for 4 hours with as little as 1 percent of this reagent gave the same test results as sizing agents as did several commercial cationic starches.

4. Flour xanthates and xanthides. During the reporting year research on xanthation and on the use of crosslinked xanthides in paper was directed primarily to studies of the products derived from starch. The results of these studies are reported under Subheading B-1 of Area 1.

Contract research on xanthates and xanthides from wheat and derived materials is being conducted by Battelle Memorial Institute. Studies have been directed to preparation and characterization of wheat bran xanthates and their evaluation as components of paper. Optimum reactant ratios for preparation of bran xanthate have been tentatively established for a process using a conical mixer rather than the Ko-Kneader type of mixer. Initial studies on stability of bran xanthates, their oxidative crosslinking and incorporation into paper, and properties of the resulting products have given encouraging results. Light and electron microscopy are being used to aid in the definition of bonding mechanisms in bran- and starch-xanthide papers.

5. Evaluation of wheat flour products for applications in the pulp and paper industry. Research on chemically modified wheat flour and related products is supported by evaluation studies to determine the quality and performance of these products in applications in the pulp, paper and paperboard industry. During the reporting period tests were conducted, for example, with enzymatically modified flour and whole ground wheat and with cationic, chemically modified flours. Results of the evaluation studies are reported in conjunction with the general discussion of research on the specific wheat flour product.

C. Processing Technology

1. Fine grinding and air classification of wheat flours. When milled and fractionated under identical conditions, 31 wheat varieties displayed an inverse relationship between protein shifting and kernel hardness as measured by the surface area of flour produced in a standardized grinding procedure. Protein shifts for the several classes of wheat averaged 6 percent for durum; 14 percent for HRS; 26 percent for HRW; 57 percent for SRW; and 62 percent for club. Impact milled flours from HRW wheat showed greater

protein shifts than conventionally milled flours, but increased ash and fat content in the flour and fractions resulted.

Engineering studies were conducted to estimate the grinding costs for conversion of mill feeds to various products. The estimated cost per ton for maximum coarseness ranging from 75 to 250 mesh were: red dog, $2.20-$4.42; middlings, $3.80-$6.84; and bran, $5.70-$8.00.

Initial studies at the University of Nebraska on crosses of soft and hard wheats of different kernel hardness have shown considerable variation in milling, air classification, and protein quality characteristics.

2. Wheat conditioning. In estimation of endosperm breakdown in flours from tempered wheat, microscopic analysis was shown to be necessary only for the fraction having particles below 30 microns in diameter. Free protein and free starch were found only in this fraction. The fraction above 30 microns contained only endosperm fragments and its breakdown was satisfactorily estimated by means of a nonselective procedure such as the Micromerograph.

Comparative microscopic sizing of wheat starches and the corresponding flours revealed that wheat having starch with a higher proportion of relatively small granules yields a flour containing more free protein particles. This result indicates that protein-starch binding is less firm in small granules formed late in kernel development than in large granules formed earlier.

3. Reduction of viable microorganisms in flour and flour products. Studies on procedures for reducing microorganisms in flour showed that the simplest method for reducing counts to less than 500 per gram is merely to store flour at 115° F. for about 10 days. Flour properties were little affected by this procedure. Continuation of microbiological examination of flours and parent wheats from mills in various locations showed that the bacterial counts of flours from the 1964 Kansas-Nebraska crop averaged about one-third lower than for the 1963 crop. Fungal counts were higher for the 1964 crop than for the 1963 crop. Bacterial counts were considerably higher for the parent wheats than for the flours milled from them, whereas the reverse order was observed for fungi. Only low levels of coliforms, fecal streptococci and lactic acid bacteria were found in flours. These findings indicate adequate mill sanitation and suggest that flour is not the principal source of lactic acid bacteria responsible for spoilage of refrigerated dough products.

4. Reduction of radioactive contamination of wheat and milled products. In the research on reducing radioactivity of wheat, a one-step washing of the grain with warm 0.5 percent citric acid solution resulted in removal of 73 percent of the Sr-90--the best results to date. This procedure has also resulted for the first time in a reduction of the residual radioactivity of patent flour. Milling studies of many samples of different varieties of wheat from various locations showed that varieties of wheat grown under identical conditions at the same location differ considerably in initial Sr-90 levels. This difference is reflected after milling by different

radioactivity in the feed fractions, whereas the radioactivity of the flour fractions was about the same. These investigations involve cooperation of the Health and Safety Laboratory of the AEC and have as their objective the development of processing methods that would yield nonhazardous wheat products in the event of a nuclear emergency. Present levels of radioactivity in wheat are well within the safe limits established by public health authorities.

5. Quantitative measurement of wheat conditioning variables. In studies under a PL 480 grant to the Cereals Research Station, Research Association of British Flour-Millers, St. Albans, England, appreciable increases in protein shifting (i.e., increased endosperm breakdown) was observed for flours milled from whole grain extracted with polar solvents. A series of tempering and milling experiments indicated that plastic deformation of the protein component weakens protein structure and leads to breakage during subsequent regrinding. Since plasticity of the protein increases with moisture content, so also does breakdown of endosperm that has undergone plastic deformation.

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AREA NO. 3: CORN, SORGHUM, AND OTHER FEED GRAINS IMPROVED INDUSTRIAL, FEED AND FOOD PRODUCTS

Problem. Abundant quantities of corn, sorghum, and other feed grains are now available beyond those amounts required to satisfy current needs. Both domestic consumption and export potential of these grains could be increased by development of new products for use by industry or of improved foods and feeds. Industrially, increased use of corn and sorghum will be mainly dependent upon increased markets for starch. However, flours derived from these grains are mixtures of starch, protein, and minor amounts of other components. Such mixtures have promise as raw materials for conversion to adhesives, water-soluble coatings, plastic materials, and related products that should have properties and uses different from related products derived from refined starch or wheat flour and that should contribute independently to increasing industrial markets. Isolated protein components of corn and sorghum flours should be suitable raw materials for production of useful resins and films. To achieve these utilization goals, more information is needed on basic physical and chemical properties and reactions of these flours, on the properties of component lipids, waxes, and proteins and their possible interactions with starch, and on the use of fine grinding and air classification and other new milling techniques for obtaining milled products having the most advantageous properties as industrial raw materials.

Because of the growing emphasis on increasing meat production, there is need for processes to obtain improved feed products such as high-protein feeds, mill feeds, feed concentrates, and feeds with high oil content. Such improvement could be achieved through research to obtain better knowledge of the biologically and nutritionally important constituents of corn, sorghum, and oats, to evaluate present, and to develop improved, milling and processing methods, and to ascertain the effects of such methods on the nutritional qualities of the products. In addition, because of the world shortage of protein in human nutrition, this research could enhance the export value of these grains by providing the necessary basis for development of high-protein and other food products that would be acceptable in foreign markets.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing long-term program involving analytical and organic chemists, chemical engineers and structural biologists engaged in basic studies of the components of corn and sorghum and in application of the new knowledge gained to the development of improved processing technology leading to more effective utilization of these cereal grains.

The Federal scientific effort for research in this area totals 8.0 professional man-years. Of this number 5.6 are devoted to chemical composition and physical properties and 2.4 to processing technology.

Research on chemical composition and physical properties (3.5 professional man-years), conducted at Peoria, Illinois, involves investigations of physiologically active nonprotein nitrogen substances in corn and of carotenoid pigments of corn, corn milling fractions, and yellow endosperm sorghum. A portion of the effort on carotenoid pigments is cooperative with Crops Research Division and is directed to development of corn and sorghum varieties having high carotenoid content. Such varieties are needed for improved food and feed products and to enhance the competitive position of U. S. corn in international trade. Research contracts (2.1 professional man-years) are in effect with Indiana University Foundation, Bloomington, Indiana, for studies on the isolation and characterization of phenolic pigments of grain sorghum (.8 professional man-year); and with Kansas State University, Manhattan, Kansas, for investigations on the composition, processing and feeding value of hybrid grain sorghums (1.3 professional man-years).

Processing technology research conducted at Peoria, Illinois, (2.0 professional man-years) involves pilot-plant studies of conditions and methods for increasing the yield of oil and grits by dry-milling processes. Effects of processing variations on industrially and biologically important components of corn are determined. A grant has been made to Pennsylvania State University, University Park, Pennsylvania, for basic studies on the mechanical and viscoelastic properties of shelled corn as related to the corn dry-milling process (.4 professional man-year).

The Department also sponsors research in this area conducted under grants of PL 480 funds to the following foreign institutions: National Institute of Agronomic Research, Paris, France, for basic studies of the physical chemical properties of corn zein (4 years, 1961-1965); and Indian Institute of Science, Bangalore, India, for research on separation of grain sorghum proteins (5 years, 1963-1968). These lines of work are under the subheading chemical composition and physical properties. During the reporting period research was completed on oat antioxidants at the Cereals Research Station, Research Association of British Flour-Millers, St. Albans, England.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations have a continuing program designed to improve the utilization of corn, sorghum, and other grains in feeds and foods. There is widespread interest in and much effort is devoted to development of varieties of grains with improved nutritive value, pigment content, or other special constituents of value in animal rations. Research on the chemical composition and physical properties of grains is being conducted to support the breeding program on the one hand, and the nutrition program on the other. Development and utilization of corn lines and hybrids with high-protein and high-oil content is an example of the work. The variation in fat and protein

content and in amino acid and fatty acid composition is being followed closely. Extraction and characterization of corn proteins permits selection of corn varieties with improved protein quality and provides information which may lead to new industrial uses.

The work on microbial problems associated with grain storage and utilization involves study of mold deterioration and its effect on wheat and corn. Biological changes are also investigated.

Handling, processing, storage and milling procedures are being researched both from the standpoint of improvement of processing procedures and from the standpoint of effects on ultimate utilization of the products. Processing treatments such as drying, pelleting, enzyme treatment, steaming, dry rolling, and pearling are being studied. Product form, such as whole, cracked, or pelleted, also affects maximum utilization of nutrients and feed efficiency. Product characteristics, such as moisture, protein and fiber content, and pigment retention are also evaluated in terms of value of the grains for feed.

The relative value of sorghum and corn for finishing beef steers is of great significance in the utilization of these grains. Effects of conditioning, drying procedures and storage conditions are important. Studies are in progress to determine the effects of processing grain-type sorghums on their utilization and nutritive value in beef and dairy rations.

Annual crop residues such as corn stalks, sorghum stems, and corn cobs provide a natural reservoir of raw materials. Studies on isolation, characterization and derivatization of hemicelluloses from these sources are in progress in an attempt to modify hemicelluloses in ways to produce new physical properties of potential usefulness.

Study of the economic feasibility and potential market expansion for selected grain crops through new uses and changed utilization patterns is in progress. Both agricultural and nonagricultural uses and particularly uses at processing and manufacturing levels are considered.

Total professional man-years involved in the utilization of cereals and other grains is 7.7

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Nonprotein nitrogen substances of corn. Studies on nonprotein nitrogen components showed that most of the nucleotides of whole corn are in the germ. However, some endosperm nucleotides did not appear in the germ. Immature corn (milky stage) contained 10 times the concentration of nucleotides as did mature corn. Trichloroacetic acid was found to be a superior solvent for extraction of nucleotides from corn. Nucleotides definitely

characterized as components of whole corn are thymidine monophosphate, guanosine di- and triphosphates, uridine diphosphate, and adenosine triphosphate. The pyrimidine nucleotides are present in largest proportion (80 percent). Sugar derivatives of guanosine and cytosine diphosphates also were identified.

2. Carotenoids and other pigments of corn and sorghum. The principal carotenoid pigments of 11 genetically different corn samples were characterized and estimated quantitatively. Total carotenoids ranged from 0 to 57 p.p.m. As carotenoid content increased, zeinoxanthin decreased; cryptoxanthin remained relatively constant as a percentage of the whole; and lutein, zeaxanthin, and polyoxy compounds increased both in absolute quantity and as a percentage of the whole. At the 57 p.p.m. level, lutein and zeaxanthin represented 70 percent of the total carotenoid content. The maximum xanthophyll level in corn breeding samples furnished by Crops Research Division during the last 6 months has increased from 72 to 77 p.p.m., an increase of 7 percent. An increasing number of samples containing more than 60 p.p.m. of xanthophyll is being observed. The average for present commercial hybrids is about 25 p.p.m.

Research at Indiana University showed that sorghum pearlings (pericarp fraction) contained most of the pigments and other phenolic compounds. Hexane-extracted wax from the pearlings contained no phenols.

3. Corn and sorghum proteins. In studies under a PL 480 grant at the National Institute of Agronomic Research, Paris, France, analysis and characterization of zein proteins was continued. During the year, particular attention was given to measurement of molecular weight, rotatory dispersion, and sulfur-containing amino acids and to the application of gel electrophoresis, gel chromatography, and amino acid analysis to protein fractions.

Research on separation of grain sorghum proteins is in progress under a PL 480 grant to the Indian Institute of Science, Bangalore, India. Protein content of endosperm (dehulled seeds) and seed coat fractions has been determined for 44 genetic strains of grain sorghum from the Rockefeller Foundation World Sorghum Collection at New Delhi. The endosperm protein content ranged from 9.2 to 19.0 percent. The endosperm of four Indian varieties which were analyzed contained from 8.5 to 10.6 percent protein. Studies on extraction of the protein by several different solvents showed the highest recoveries were obtained with alcoholic alkali. Research on the detection and separation of component proteins has been initiated.

4. Antioxidants in oats. The identity of one group of phenolic antioxidants naturally present in oats has been established. These compounds have been identified as diesters of caffeic or ferulic acid with C-26 or C-28 long-chain fatty diols. A U. S. patent (assigned to the Department) has been granted on these antioxidants, which are comparable in activity to

propyl gallate. This research, now completed, was conducted under a PL 480 grant at the Cereals Research Station, Research Association of British Flour-Millers, St. Albans, England.

B. Processing Technology

1. Corn dry milling. In studies on tempering low-moisture corn, it was found that temper times of 1 hour or more were needed for absorption of appreciable moisture by the germ and adjacent endosperm but not for good degermination. The principal value of tempering was in reduced production of fines and increased recovery of oil. A second temper mainly facilitated good hull removal. Preheating low-moisture (old or artificially dried) corn and temper water to 115° F. or higher before mixing or tempering with open steam practically eliminated stress-crack formation. Without prior warming, about half the kernels developed fissures, with resultant adverse effects on yield and oil content of large grits. A variety of samples has been provided for study under the grant at Pennsylvania State University on mechanical and viscoelastic properties of corn kernels.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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*Research supported by PL 480 funds.

AREA NO. 4: HIGH-AMYLOSE CORN INDUSTRIAL UTILIZATION

Problem. Varieties of corn have been achieved genetically that contain greatly increased amounts of amylose. Amylose, the linear fraction of starch, possesses film- and fiber-forming properties not available in ordinary starch which contains only about 27 percent of this component. Because the unique properties of amylose open areas of utilization closed to ordinary starch, the potential industrial value of this new crop is very high. Several problems must be solved, however, to realize this potential.

For high-amylose starch to have substantially improved properties as a raw material in comparison with ordinary starch, it should contain at least 80 percent of amylose. Many breeding samples have recently been observed that contain over 80 percent of amylose. However, only varieties containing up to about 75 percent have so far been commercially available. Over 5 million pounds of high-amylose starch from commercial plantings are now utilized annually by industry. Although breeding is the task of the geneticist, utilization research is needed to provide information on amylose content, on changes in quantities and properties of the amylose, amylopectin, and other components such as oil and protein, and on milling characteristics of breeding samples in order to insure availability of satisfactory varieties.

To insure utilization of the potentially large volume of high-amylose starch that could eventually become available, more information is needed on the chemical and physical properties of amylose and high-amylose starch and on methods for converting them economically to desired products. Success in this research could lead to an estimated consumption of over several hundred million pounds of high-amylose starch by 1975 in plastics, coatings, films, fibers, and related products to which the linear character of amylose could make contributions.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a long-term, continuing program of research involving analytical, organic and physical chemists, structural biologists, and chemical and mechanical engineers who are engaged in basic and applied research designed to increase knowledge of the properties and reactions of amylose and other components of high-amylose corn and to utilize this knowledge in development of attractive industrial applications for amylose and high-amylose starch.

The Federal scientific effort for research on utilization of high-amylose corn totals 13.1 professional man-years. Of this number 10.7 are devoted to chemical composition and physical properties and 2.4 to industrial utilization.

Research at Peoria, Illinois, on chemical composition and physical properties (9.1 professional man-years) involves study of amylose content of breeding samples, starch and starch granule composition, structure and properties; and composition and properties of proteins and other components of high-amylose corn. Studies on amylose content of breeding samples assist geneticists in developing varieties of high-amylose corn having increased amylose content. Cooperation with Field Crops Research Branch, Crops Research Division, is maintained in conducting these studies. A research contract (.2 professional man-year) is in effect at Arizona State University, Tempe, Arizona, for basic research on the interaction of "V" amylose with small organic molecules. Grants (1.4 professional man-years) have been made to the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for basic studies on variations in starch granules of genetically different corn samples (.8 professional man-year); and to Princeton University, Princeton, New Jersey, for basic research on the relationship of viscoelastic properties of amylose film to structure and function of plasticizers (.6 professional man-year).

Research on industrial utilization, which is conducted at Peoria, Illinois, is devoted to preliminary studies of chemical and physical modification of high-amylose starch as means for formation of amylose films having industrially acceptable properties (2.4 professional man-years).

PROGRAM OF STATE EXPERIMENT STATIONS

The station phase of the program designed to develop high-amylose corn for industrial uses is largely one of support for the breeding program. The Indiana, Missouri, and Nebraska stations are continuing development of lines high in amylose content. Samples are analyzed, often on an individual plant basis, to determine amylose content. Some additional effort is devoted to development of techniques or processes for separating amylose from other kernel constituents, to study of enzyme systems, and to search for industrial applications.

Total effort devoted to high-amylose corn utilization is about 1.0 professional man-year.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Amylose content of breeding samples. During the reporting period 16,875 samples of high-amylose corn were analyzed. These were supplied under Memoranda of Understanding by the Bear Hybrid Corn Company, by the Missouri Agricultural Experiment Station and by Crops Research Division at the Missouri Station. Several thousand samples contained over 80 percent amylose. Among the samples analyzed during the last 6 months of the period were three samples at the 85-percent level, a new high in development of high-amylose corn. (In this report, percentages of amylose refer to

apparent values determined by iodine sorption. True amylose content, measured by quantitative fractionation, averages about 80 percent of the apparent value. According to reports from industry, this distinction has so far not proved significant in industrial utilization of high-amylose starch.)

In view of the attainment of strains having starch of 85 percent apparent amylose content, there is optimism that further progress will be made and belief that the limit of amylose content in high-amylose corn is not yet in sight. Limited quantities of Class 7A amylomaize (70-75 percent apparent amylose) have been grown by Bear Hybrid Corn Company and supplied to several processors for research purposes. It is anticipated that beginning with the 1967 planting season, Class 7 will be available to any processor requesting it.

2. Properties of components. The existence of protein-carbohydrate complexes involving amylopectin has been confirmed. In fact, it appears that such complexes exist in both the dispersed and granular state of amylopectin in dent and waxy corn. The complexes can be destroyed by phosphate buffer (pH 7), by hydrolysis at pH 4 and 100° C. or by action of the proteolytic enzyme Pronase. Based on the action of Pronase, a practical laboratory procedure has been developed that permits quantitative isolation of corn starch without degradation or alteration of the molecular structure of the starch components. Molecular weights of amylopectins from dent and 70-percent HA starches isolated by the new procedure are extremely high--1 billion and 175 million, respectively. The procedure is suitable for very small samples; e.g., starch content can be determined on as little as four kernels.

The availability of unaltered starch as provided by this new method of solution will greatly facilitate basic studies on starches from corn genotypes and should lead to much more accurate information on the properties and structure of these starches.

Contract research on V-amylose at Arizona State University was directed to investigation on the stability of V-amylose and V-amylose hydrate in equilibrium with salt-hydrates of known vapor pressure. At or above 50° C., the hydration reaction was reversible. The heat of reaction of water with amylose was calculated to be 9 k. cal. per mole of water. V-Amylose was reacted with gaseous and liquid NH_3 to form stable compounds. The reaction with liquid NH_3 is irreversible, suggesting that the NH_3 has penetrated to the interior of the helix and is tightly bound. The product retained the helical structure and was soluble in hot water.

Research on granule variation under the grant to the Nebraska AES indicates that water absorption of starch of the waxy genotype (wx) is much higher than that of starch from the sugary genotype (su_2). Starch governed by the combination su_2wx displayed an intermediate level of water absorption.

3. Molecular properties of amylose film. Molecular organization in amylose films cast from solutions in dimethyl sulfoxide (DMSO) was found to vary with the conditions of evaporation of the solvent. At 0-30 percent relative humidity amorphous structure resulted, as shown by X-ray diffraction. At 40-93 percent relative humidity, B and V structures were formed. Amorphous films could be converted to B-type by wetting with water and drying at room temperature. Heating amorphous films in a butanol-water mixture produced V-type structure. Agents used to plasticize amylose films, such as glycerol and ethylene glycol, formed recognizable complexes with the amylose.

Research on the mechanism of external plasticization of amylose films has been initiated under the grant to Princeton University.

B. Industrial Utilization

1. Chemical and physical modification of high-amylose starch. Treatment of HA starch with DMSO increased its reactivity to acid chlorides and anhydrides and other derivatizing reagents. Certain esters were soluble in hot water. Hydroxypropylated DMSO-pretreated HA starch (D.S. above 0.03) gave films soluble in hot or cold water. Unplasticized films had a tensile strength of 10,129 p.s.i., elongation of 11.4 percent and MIT fold of 523. Initial experiments showed that oxygen transmission of plasticized and unplasticized films was below the limit of measurement at 5° and 25° C. over a wide relative humidity range. Studies are planned to confirm these findings. The properties of these low D.S. products suggest that the cost of chemically modifying HA starch as a means of obtaining new or improved films or other products may turn out to be nominal.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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Mark, A. M., Roth, W. B., Zobel, H. F., and Mehltretter, C. L. 1965. Properties of dimethyl sulfoxide-pretreated amylomaize starch. Cereal Chem. 42(2), p. 209.

AREA NO. 5: WHEAT AND CORN
FERMENTATIVE CONVERSION TO NEW INDUSTRIAL, FEED AND FOOD PRODUCTS

Problem. By fermentation of cereal grain substrates, new products can be obtained that are not readily available by other means and have promising potential for industrial, agricultural, and food uses. Processes now under development, if brought to successful conclusion, could lead to substantially increased consumption of grain for fermentative conversion to stable viscosity agents for secondary petroleum recovery by flooding of spent oil wells, to new organic acids and enzymes for industrial use, to feed supplements, and to effective biological insecticides and other pesticides that are harmless to man. In addition, there are good possibilities for utilizing fermentation processes to produce new food products that should promote foreign use of U. S. grains.

To accomplish these objectives and to realize the full potential of fermentative techniques for increasing utilization of grain, a broad program of exploratory research is required to find and identify through taxonomic studies species of organisms producing potentially valuable products, to isolate high-yielding strains or develop them by mutation, hybridization or genetic selection, and to develop basic information on culture media, special nutrients, and other factors required for optimum growth of microorganisms and maximum yields of desired products. Continued maintenance and expansion of a collection of pure cultures of well-characterized organisms is necessary for this research. For successful translation of laboratory results into commercially useful processes, more information is needed on new techniques of fermentation, on development of economical methods of growing organisms and handling fermentation processes on a large scale, and on special procedures for efficient isolation and purification of products from fermentative reaction mixtures. Finally, the most appropriate end uses for products must be identified and information obtained on product evaluation and development.

USDA AND COOPERATIVE PROGRAMS

The Department has a long-range continuing program involving analytical and organic chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic research on microorganisms and microbiological reactions and products and in application of both known and newly discovered principles to the development of practical fermentation processes for conversion of cereal grain substrates to useful chemical, biological, feed and food products.

The Federal scientific effort in this area of research totals 67.6 professional man-years. Of this number 29.4 are devoted to basic research on fermentation processes, 21.1 to industrial chemicals, 11.8 to biological pesticides, and 5.3 to feed and food products.

Basic research on fermentation processes conducted at Peoria, Illinois, (27.3 professional man-years) includes study of taxonomy of molds, yeasts and bacteria; factors affecting viability of microorganisms; and microbiological reactions and products. Because of the importance of the problem, the research effort on mycotoxins has been greatly intensified. Basic to these investigations and to the Division's entire research program on fermentation is assembly and maintenance in pure culture of a large collection of agriculturally and industrially important microorganisms. Much of the research on microbiological reactions and products is conducted by the Pioneering Laboratory for Microbiological Chemistry. During the reporting period taxonomic research on streptomycetes was restricted to studies required in connection with antifungal antibiotics. A research contract (.2 professional man-year) with American Type Culture Collection, Rockville, Maryland, provides for studies on preservation of certain microorganisms for which lyophilization is ineffective. A grant has been made to Iowa State University, Ames, Iowa, for investigation on bacterial amylases and their action patterns (1.9 professional man-years).

Research at Peoria, Illinois, on industrial chemicals (19.5 professional man-years) involves fermentative production of microbial gums, organic acids, and other products for use in the chemical industry. This work includes investigation and development of improved or new procedures for conducting industrial fermentations. During the reporting period studies on enzymatic modification of wheat flour were completed and replaced by research on enzymatic conversions of starch and glucose to products of possible industrial value. During the reporting period contract research on polymerization of selected fermentation acids and derivatives of fatty acids was completed at the University of Arizona, Tucson, Arizona. Grants (1.6 professional man-years) have been made to Cornell University, Ithaca, New York, for fundamental studies on biphasic fermentation (.8 professional man-year) and to the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on the nature of amylase enzymes (.8 professional man-year).

Research at Peoria, Illinois, on biological pesticides (9.1 professional man-years) is devoted to studies on biological insecticides for Japanese beetle and on antifungal antibiotics for use in control of economically important plant diseases. Investigations on biological insecticides for Japanese beetle and on other insect control agents is cooperative with Entomology Research Division and Plant Pest Control Division. Research on plant antibiotics involves cooperation with Crops Research Division. During the reporting period screening studies to find new antifungal antibiotics were completed and redirected toward practical evaluation of several new antibiotics revealed by the screening program. Research contracts (2.7 professional man-years) covering various phases of research on Japanese beetle pathogens are in effect at Michigan State University, East Lansing, Michigan, for basic research on enzyme activity in sporulation (.7 professional man-year); at Kansas State University, Manhattan, Kansas, for investigation of stabilization of vegetative cells of the pathogenic

organisms (.5 professional man-year); at the University of Minnesota, St. Paul, Minnesota, for fundamental studies on the transfer of genetic determinants of sporulation from one microorganism to another (.5 professional man-year); at the University of Illinois, Urbana, Illinois, for research on the applicability of a sporulation factor produced by bacteria to Japanese beetle pathogens (.6 professional man-year); and at Baylor University, Houston, Texas, for investigation of morphological changes involved in sporulation (.4 professional man-year). During the reporting period contract research providing for study of factors important to large-scale production of Japanese beetle pathogens was completed at Michigan State University, East Lansing, Michigan.

Research at Peoria, Illinois, on feed and food products (2.8 professional man-years) involves study of production of microbial carotenoids suitable for feed supplements and development of new fermented wheat foods that can help increase export markets for U. S. wheat. Research contracts (2.5 professional man-years) are in effect with A. D. Little, Inc., Cambridge, Massachusetts, for studies on stabilization of fermentative β-carotene (1.7 professional man-years); and with Consolidated Laboratories, Inc., Chicago Heights, Illinois, for research on the use of antimetabolites to facilitate selection of higher yielding strains of microorganisms producing β-carotene (.8 professional man-year).

The Department also sponsors research in the fermentation area conducted by foreign institutions under grants of PL 480 funds. Basic research on fermentation processes involves grants to the University of Milan, Milan, Italy, for basic studies on the metabolic pathway to 5-ketogluconic acid in Acetobacter species (5 years, 1961-1966); University of Allahabad, Allahabad, India, for collection of new Mucorales species (5 years, 1961-1966), and studies on survival of lyophilized microorganisms (5 years, 1962-1967); University of Durham, Newcastle-upon-Tyne, England, for investigations of sugar phosphate derivatives in molds (5 years, 1962-1967); Central Drug Research Institute, Lucknow, India, for studies on aerobic actinomycetes in India to find new accessions for the ARS Culture Collection (5 years, 1965-1970); and to the University of Liege, Liege, Belgium, for research to find lytic enzymes of microbial origin (5 years, 1964-1969). During the reporting period the project on Pseudomonas conversion of glucose at the Indian Institute of Science, Bangalore, India, was cancelled because adequate staffing could not be achieved. Research was completed on collection of new species of yeast at the National Institute for Agronomic Research, Madrid, Spain, and on organic phosphorus compounds of yeast at the University of Helsinki, Helsinki, Finland.

Research on industrial chemicals involves grants to the University of Lodz, Lodz, Poland, for research on the fermentative production of itatartaric acid (5 years, 1963-1968); University of Tokyo, Tokyo, Japan, for research on the fermentative production of D-tartaric acid (5 years, 1964-1969) and

of mevalonic acid (3 years, 1965-1968); and University of Baroda, Baroda, India, for studies on production of microbial lipases useful for modifying vegetable oils (5 years, 1965-1970).

Research on feed and food products involves a grant to the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, for research on production of vitamin B_{13} (5 years, 1960-1965); and the National Institute for Agronomic Research, Paris, France, for studies on mutation of yeasts for improved feeds (3 years, 1963-1966).

PROGRAM OF STATE EXPERIMENT STATIONS

The Montana station is studying conversion of barley into feed yeast protein. Barley carbohydrates are converted enzymatically into fermentable sugars which, in turn, serve as an energy source for the yeast. Current work involves establishing reliable yield and cost data for analysis of the process. In fermentation studies at other stations, work is directed toward biosyntheses of lysine and of polysaccharides, for example. Still other research involves fundamental studies of the organisms, the fermentative process and methods for separating desired products from fermentation liquors.

The total research effort devoted to fermentative conversion as a means of utilization is 2.2 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Basic Research on Fermentation Processes

1. ARS Culture Collection. As of January 1, 1965, the ARS Culture Collection contained 16,934 permanent cultures, an increase of 2,863 over 1964. By way of comparison, the number of permanent cultures on January 1, 1960, was 8,883. The permanent collection has, therefore, nearly doubled in 5 years. During 1964 a total of 2,254 cultures were distributed to domestic and foreign recipients.

Contract research by the American Type Culture Collection revealed that 47 of 54 cultures (about 50 species) of molds tested remained viable after freezing and storing under liquid nitrogen for 2 weeks. This recently initiated work is designed to determine if the liquid nitrogen procedure can preserve organisms that do not survive lyophilization and have required serial transfer methods of maintenance.

Research of importance to the ARS Culture Collection is in progress at several foreign institutions under PL 480 grants. At the National Institute of Agronomic Research, Madrid, Spain, studies on collection and characterizing new strains of yeasts have been completed. More than 100,000 specimens were examined, and 1,399 cultures were characterized. A total of 1,023 yeasts were received and incorporated into the ARS Culture Collection. At

the University of Allahabad, Allahabad, India, results indicated that lyophilization of spores of Aspergillus niger did not adversely affect the production of citric acid. Also, there appeared to be a small decrease in viable spores as the lyophil preparations aged. Other research at the University of Allahabad is continuing to supply the Northern Division with many new isolates of Mucorales. A number of these cultures are being used in the in-house taxonomic studies on Rhizopus (see item 2, following).

2. Taxonomic investigations. A family of yeasts, the Chlamydozymaceae, has been erected based upon discovery of a highly developed and complex type of sexuality in an abundantly common group of yeasts whose asexual forms have been known for many years. The gametes consist of two sexes and the sexual reactions are strong, in some cases being driven by sexual agglutination. The distinguishing characteristic of protosexuality is the rapid conversion of bisexuals to unisexuals without an intervening stage that consists of sexual spores. These observations suggested the prediction that genera of protosexual yeasts would be found which had given rise to primitive ascomycetes and basidiomycetes at the taxonomic interface between the protosexual yeasts and two main classes of fungi that produce respectively ascospores and basidiospores. A very interesting species that occurs near this taxonomic interface was studied. The most primitive heterothallic species of Hansenula yet discovered, Hansenula wabatongushiensis, was found to have two sexual cycles, one ascosporogenous, the other protosexual. The two phases are separately possessed by certain genetically different isolates.

In studies on Mucorales, new zygosporic strains have been found in two more heterothallic species in the section Dubiorugorhizopus of the genus Rhizopus. A (-) strain of R. niveus was found to give an imperfect mating with a (+) strain of R. oligosporus (the tempeh-producing organism). There are also one (-) strain of R. oligosporus and 12 (+) strains that can be successfully mated to give many mature zygospores. These studies open possibilities for investigation of the effects of mating strains, sexual strains and possible hybridization on tempeh production and flavor.

The scope of basic taxonomic studies of streptomycetes was reduced soon after the start of the reporting year. In further investigations along these lines, emphasis is being placed on the taxonomy of special groups of antibiotic-producing organisms (see Section C, following).

3. Microbiological processes and products. In studies on genetic control of fermentation, omission of glucose from the medium or growth at 40° C. decreased but did not eliminate undesired autolysis of Pseudomonas aeruginosa. Several clones derived from one strain by combination of growth at 40° C. and exposure to acriflavin appeared to be free of autolysis. Attempts to secure conjugation between a male parent (strain 1093) and the female parent (1090) resulted in a preferential kill of the male parent. If mating was conducted at 40° instead of 28°, this kill became negligible.

A standard procedure was devised for evaluating the capacity of microorganisms to modify fatty acids fermentatively. Preliminary results indicated that degradation occurred with increasing difficulty as the chain length of the fatty acids increased. With availability of an effective method for evaluating the ability of microorganisms to modify fatty acids, accelerated progress is expected in identifying conversions of potential value to utilization research.

In the Pioneering Laboratory for Microbiological Chemistry, phosphatidyl glycerol and phosphatidyl ethanolamine were tentatively identified as two of three phospholipid fractions isolated from particles derived from bacterial cytoplasmic membranes and probably associated with electron transfer reactions in cell membranes and organelles of high metabolic activity. Studies on the macromolecules responsible for agglutination of certain yeast strains resulted in preparations with very high specific activity (agglutination achievable with 1.7 particles per yeast cell; 1.0 particle is the theoretical minimum). Specific activity increased with molecular weight over the range of 1 million to several hundred million. An acid phosphatase from Saccharomyces mellis was shown to be an exocellular enzyme. Research on the chemistry of compounds of microbial origin has shown that the acetylated hydroxy acid produced by an unidentified yeast is 8,9,13-triacetoxydocosanoic acid.

In other work in the Pioneering Laboratory, the fungus Sclerotinia sclerotiorum was utilized as a model system for study of composition, metabolism and control mechanism involved in morphological development of sclerotia (hardened masses of mycelia resistant to unfavorable environmental conditions). Analysis of sclerotia of S. sclerotiorum showed that they are low in fat but contain large quantities of carbohydrate and protein. Several of the carbohydrates were identified. When sclerotia are placed in a suitable medium, they may either resume vegetative growth or develop sexual reproductive structures. Information was obtained on procedures for inducing the reproductive structures, on carbohydrate utilization by strains of S. sclerotiorum, on an extracellular polysaccharide produced in laboratory cultures, and on the biosynthetic pathway to D-mannitol in growing mycelium.

In studies under a PL 480 grant at the University of Milan, Milan, Italy, enzymes responsible for formation of 2-ketogluconate were found to be partly constitutive, whereas those producing 5-ketogluconate were inducible. Fermentations yielding primarily 2-ketogluconate may be possible if the basis for repression of the 5-ketogluconate enzyme system can be determined and controlled. Fermentations to yield only 5-ketogluconate appear to be impossible except by mutational loss of the constitutive 2-ketogluconate system.

At the University of Helsinki, Helsinki, Finland, all of the major metabolically active phosphate esters of yeast that accumulate or that are rapidly labelled with radioactive phosphate have now been identified.

Accumulation of trehalose phosphate in both Candida utilis and Saccharomyces cerevisiae was temperature dependent and enhanced by aeration. These observations suggest the presence of a thermally induced enzymic block. This research is being conducted under a PL 480 grant. Laboratory work has been completed, but the final report has not yet been received.

Studies recently undertaken under a PL 480 grant to the University of Liege, Liege, Belgium, have revealed conditions for obtaining high, reproducible, bacteriolytic activities in culture filtrates of Streptomyces albus G. A procedure for isolation and fractionation of the "F_1" enzyme complex, which attacks several bacterial species resistant to lysozyme, has been developed. The F_1 muramidase has been separated from at least three less basic peptidases which have different pH optima and are species specific with regard to bacterial substrate.

Studies on microbial sugars, conducted under a PL 480 grant at the University of Durham, Newcastle-upon-Tyne, England, indicate that the ribitol teichoic acid from Streptomyces griseus is composed of ribitol residues joined through phosphodiester linkages. β-Glucopyranosyl substituents are attached to some of the ribitol units. The nucleotides in S. niveus have been identified as uridine diphosphate (UDP), UDP-glucose and a mixture of UDP-N-acetylglucosamine and UDP-N-acetylgalactosamine with a labile group attached to the amino sugar.

4. Aflatoxin investigations. Screening studies have revealed three strains of Aspergillus flavus to be the best aflatoxin producers among 22 strains tested. Aflatoxin could be produced on either still or shaken broths. Cracked corn, rice, wheat, sorghum and soybean grits were tested as substrates for aflatoxin production. Rice proved to be the best solid medium for producing consistently high yields of aflatoxin. The poorest medium was soybean grits with or without added methionine.

In the survey of commercial grains for incidence of aflatoxin, a total of 1,382 samples have been received and 347 samples from the poorest grades have been screened for aflatoxin. Positive results were obtained for 5 samples of sorghum, 3 of corn, and 11 of oats. Negative results were obtained for wheat. A number of soybean samples contained significant amounts of a fluorescing factor that proved not to be aflatoxin. These results are perhaps not unexpected since the low grades of grain tested are those to which moldy and damaged grains are assigned. Any conclusions regarding the incidence of aflatoxin in grains must await completion of the survey and study of data for the highest grades of grain. Growth of some species of the genus Bacillus and several Clostridia was inhibited when 10-20 µg of 33 percent pure aflatoxin per ml. of substrate was present.

B. Industrial Chemicals

1. Conversion of grains to fermentation media. A second strain of A. niger that produces high yields of glucamylase has been discovered. Formation of

transglucosylase is lower for this new strain than for the strain reported last year. Experiments with this earlier strain showed that amyloglucosidase yields of 10 to 12 units/ml. could be obtained in 20-liter fermentors with 20 percent corn slurry as substrate.

The organism Candida tropicalis Y-1367 was found to produce a transglucosylase capable of effecting reaction between starch or dextrin and glycerol. This enzyme could prove useful for large-scale production of glycerol glucoside.

2. Enzymatic modification of wheat flour. Tests of enzymatically modified ground whole wheat products showed that acceptable paper sizes could be obtained. Apparently because the action of the enzymes was affected by the presence of the bran, conditions for modification were markedly different from those required for flour. Settling of bran also constituted a problem. These investigations have been completed. Future effort will be directed to research on enzymatic conversions of starch and glucose to products having possible industrial value.

3. Studies on fermentation techniques. A two-stage stirred reactor comprising a 30-gal. fermentor in series with one of 60-gal. capacity was successfully used for a 6-day continuous fermentation to produce polysaccharide B-1459 in an overall yield of 42 percent. The product was of good quality, but foaming and viscosity characteristics indicated the possibility of physiological change in the culture. The polymer synthesized in the present studies has a lower uronic acid content and may be different in either molecular size, unit distribution or branching from batch-produced material.

Initial research on biphasic fermentation at Cornell University involved studies on the toxicity of water-immiscible alcohols to bakers yeast. In general, toxicity was proportional to solubility. Attempts were made to grow the B-1459 organism on membranes, but the growth and polysaccharide formation were not sufficiently better than in shake flasks to justify further study of this system.

4. Screening and structure studies on microbial polysaccharides. Gas chromatographic procedures have been developed for quantitative (± 1.0 percent) resolution of mixtures of anhydroglucoses and of hexoses and pentoses. Phosphogalactans from Sporobolomyces sp. have been shown to contain galactose, phosphate and O-acetyl in the approximate molar ratio of 9:1:1. The phosphogalactosyl groups are terminal and permit galactose to be split off easily, leaving the monophosphate which still retains viscosity.

Studies on ultracentrifugal determination of the molecular weight of polysaccharide B-1459 revealed that molecular aggregation is the cause of difficulty in applying this method.

Toxicity of B-1459 was investigated in the Pharmacology Laboratory of the Western Division. No unfavorable effects were observed. Safety evaluation data were obtained with rats and dogs for inclusion in industrial petitions to Food and Drug Administration pertaining to a proposed use of the polysaccharide as a food-thickening agent. Approval for food use would open new, large outlets for B-1459.

5. Fermentation acids. In studies under a PL 480 grant to the University of Lodz, Lodz, Poland, strains of Aspergilli were mutated to increase yields of itaconic acid, but no strains capable of direct conversion of glucose to itatartaric acid were reported. However, the conversion of itaconic acid to itatartaric acid is a simple step that can be accomplished by an oxidative dehydratase.

Other research on fermentation acids, recently undertaken under PL 480 grants to the University of Tokyo, Tokyo, Japan, includes studies on production of D-tartaric acid and investigations on formation of mevalonic acid. No significant results have yet been reported.

C. Biological Pesticides

1. Biological insecticides for Japanese beetle. Substances that might be expected to preserve cell wall integrity, such as trypsin inhibitor, nucleic acid and acetyl choline, have increased sporulation of B. popilliae on solid culture media. Content of borate in the medium was found to influence sporulation significantly. Increased borate levels stabilized cells but decreased sporulation presumably by decreasing cell wall permeability. A technique for the isolation of whole cell walls of B. popilliae has been devised. Free spores of this organism were also isolated and characterized. Extensive experiments indicated that B. popilliae cultures do not carry a demonstrable phage even though minute lytic areas have sometimes been observed in test plates inoculated with ultraviolet-irradiated B. popilliae.

Additional information was accumulated on the composition of hemolymph from healthy and diseased grubs and on improved media and methods for increasing cell populations of cultures. Grubs have been successfully obtained by breeding of adult Japanese beetles in the laboratory.

Contract research on artificial culture of milky disease bacteria has been completed at Michigan State University. Media and conditions for producing cell populations up to 3 billion per ml. (biphasic) and viability up to 14 days were developed. An unexplained need for barbituric acid in synthetic media was discovered. A sporulation medium containing this ingredient caused 50-100 percent of the cells to develop sporelike bodies. Other important findings point to the possible importance in sporulation of acetate metabolism and intracellular accumulation of hydrogen peroxide. Other contract studies at Michigan State University revealed that ribosidase activity was greater in in vitro "spore-bodies" than in vegetative cells, but no activity was observed in spores developed in diseased grubs. Up to 78 percent of

"sporelike" bodies were obtained by addition of β-hydroxybutyrate to cultures on the third day of incubation. Supplemental feeding of glucose and L-arginine to the medium resulted in total cell populations of 4 X 10^9/ml. at 48 hours of incubation and 80 percent "sporulation" by the 16th day of incubation.

Studies on the sporulation factor at the University of Illinois showed that addition of a sporulation factor isolated from several strains of B. cereus, popilliae and lentimorbus to a growing culture of B. popilliae damaged the cells but did not induce sporulation. This uncharacterized sporulation factor was found both in B. popilliae strains that fail to sporulate on solid medium and in the strain that does. These observations indicate that failure to sporulate is due to some sort of cell malfunction but not to absence of any recognized sporulation factor. The sporulation factor, now named "sporogen," has been isolated in pure crystalline form and partially characterized. Only 19 mg. was recovered from 20 pounds of B. cereus cells.

Scientists at the University of Minnesota have produced and isolated auxotrophic mutants of B. cereus T which will serve as a source of transforming DNA in transfer experiments.

At Baylor University College of Medicine, electron microscopy of thin sections of B. popilliae cells showed a 3-layered cell wall, which is unique among gram-positive bacteria, and has provided other extremely detailed information on the fine structure of spores of B. popilliae. Fluorescence microscopy showed that old cells of B. popilliae experience leakage of cell contents.

Studies on stabilization of vegetative cells of B. popilliae are in progress at Kansas State University. Some success has been achieved in devising systems that prolong cell life as judged by plating methods. However, no system has yet proved effective for preservation of cells in soil.

The success in achieving high percentages of "sporelike" bodies suggests that the sporulation problem may now be beginning to yield to the massive scientific effort applied against it. Of first importance, however, is to determine if these "sporelike" bodies are in fact spores and, if not, what their relationship is to true spores. The characterization studies on spores at the Northern Division and the electron microscopic studies at Baylor University provide the basis for differentiating true from imperfect spores.

2. Plant antibiotics. An additional [illegible] streptomycetes were found that yielded promising nonpolyenic antifungal activity. Trials of 20 of the most promising antibiotics against 12 plant diseases and 1 nematode infestation are now in progress at Boyce Thompson Institute. These trials are being conducted on host plants so that the results when available should provide definitive indication of the value of the antibiotics tested.

D. Feed and Food Products

1. Microbial carotenoids. In runs in 20-liter fermentors, a maximum wet-cell weight of 19 g. per 100 ml. of medium has been obtained with Chlorella "vulgaris." This organism produces xanthophylls, mainly lutein and zeaxanthin. The present wet-cell weight is equivalent to 3-6 mg. of xanthophyll (dry basis) per 100 ml. of medium. A tenfold increase in yield would, however, be needed to have a process justifying industrial interest. Ten pounds of a culture of another promising alga, Chlorella variegatus, was produced in 60-liter fermentors for more detailed study. At glucose concentrations of 3 to 10 percent, chlorophyll formation is inhibited and xanthophylls (about 75 percent lutein) are the predominate product. Resting cells of some 200 molds, yeasts and bacteria failed to convert β-carotene to xanthophyll.

Contract research by the Arthur D. Little Company on stabilizing fermentation β-carotene showed that the half-life toward oxidation could be extended many months by embedding the β-carotene in gelatin containing a carbohydrate such as dextrose. A polymer precipitation technique has provided limited success, but conventional coating techniques have so far been unsuccessful.

Contract research by Consolidated Laboratories, Inc., on use of antimetabolites to develop high-yielding strains of the β-carotene-producing microorganisms has been initiated but no results have been reported.

2. Fermented wheat foods. Tempeh fermentations with wheat as substrate were found to be more susceptible to contamination by undesired microorganisms than those based on rice or soybeans. Present results suggest that fermentation at low pH's may prove to be an effective means of minimizing bacterial contamination. At the same time, low pH may provide the further advantage of favoring a better or more complete fermentation.

Partially purified proteolytic enzymes produced by Rhizopus oligosporus NRRL 2710 (the organism used for making tempeh from wheat) was found to be 3 to 5 times more potent than pepsin. Activity was particularly high toward wheat gluten. Study of enzymes involved in production of fermented wheat food products is providing new information that should have application in developing such foods and that has important implications in relation to enzyme chemistry and technology.

3. Vitamin B_{13}. In research under a PL 480 grant at the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, fractions have been isolated from dried distillers' solubles comprising peptidic substances, at least seven phenols, carboxylic acids and carbonyl compounds. Characterization of the fractions is in progress. No single component isolated thus far produces a growth response in test animals like that obtained with concentrates of distillers' solubles.

4. Improved feeds by mutation of yeasts. Under a PL 480 grant to the National Institute of Agronomic Research, Paris, France, studies are in progress that have as their objective selection and mutation to obtain strains of yeast capable of producing high quantities of sulfur-containing amino acids for use in improvement of cereal-based feeds deficient in these amino acids. Mutant strains of the yeasts Candida utilis and C. tropicalis were obtained from cells resistant to a mutating agent, ethionine. Cells of the C. utilis mutants revealed a 10-percent net increase in intra- and extracellular methionine. There was negligible increase in the cystine content of the cells, although these and all other mutated C. utilis cells contained more nitrogen than the parent strains. The increases in nitrogen and particularly in methionine are considered significant.

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AREA NO. 6: FLAXSEED
INDUSTRIAL UTILIZATION OF LINSEED OIL

Problem. Traditional markets for linseed oil, the major drying oil produced and used in the United States, are threatened by widespread use of synthetic products derived from nonagricultural sources. Thus, over the years 1950-1960, use of linseed oil in drying oil products decreased from 590 to 351 million pounds because of displacement by synthetic materials capable of better performance. During the same period, consumption of synthetic products in protective coatings increased by 50 percent.

To restore the competitive position of linseed oil, new or expanded markets are urgently needed. Such markets can be achieved by an adequate program of basic and applied research. Recent studies by Department scientists have resulted in commercial manufacture and sale of linseed emulsion paints for exterior use that are competitive with synthetic resin emulsion paints. Expanding use of these new linseed oil paints is helping to maintain linseed oil in the market for exterior paints, which amounted to 70-75 million gallons in 1962. Another new product from linseed oil to which Department research is contributing is a protective coating for concrete that prevents deterioration from de-icers and freezing and thawing in winter. Additional research is needed to insure maximum acceptance and consumption of these new coatings and to provide still other new or improved products from linseed oil that can maintain and increase its use in all types of protective coatings, a market amounting to over 700 million gallons in 1964.

Other new outlets can be realized by chemical modification of linseed oil to obtain materials that will find applications in the multibillion-pound annual market for products of the organic chemical industry. To furnish a sound basis for chemical modification, a broad program of basic research on linseed oil is required to furnish new leads and new concepts that will point the way to those products having the best chance for acceptance.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing long-range program involving analytical, organic and physical chemists and chemical engineers engaged in basic research on the chemical reactions of linseed oil and its component fatty acids and in the application of the knowledge gained to the development of new or improved products for the chemical and protective coating industries.

The Federal scientific effort concerned with research on industrial uses for linseed oil total 17.8 professional man-years. Of this number 4.4 is devoted to industrial chemical products and 13.4 to protective coating products.

The current program at Peoria, Illinois, does not include research specifically devoted to chemical composition and physical properties of linseed oil.

Research at Peoria, Illinois, on industrial chemical products (4.4 professional man-years) involves exploratory studies to find new reactions and chemical derivatives and basic and applied research on cyclic fatty acids.

Studies on protective coating products in progress at Peoria, Illinois (10.4 professional man-years), include investigations on new resins and polymers from linseed oil for use as coatings and water-soluble vehicles, and basic research on problems related to development of linseed emulsion paints and to durability of linseed oil films. Research contracts on protective coating products (3.0 professional man-years) are in effect with Kansas State University, Manhattan, Kansas, for research on the use of linseed oil to protect concrete (.7 professional man-year) and on its use as a single coating for both curing and protection of concrete (.9 professional man-year); with North Dakota State University of Agriculture and Applied Science, Fargo, North Dakota, for investigations of aldehyde oils as components of protective coatings (.4 professional man-year); and with Stanford Research Institute, Menlo Park, California, for studies on properties and reactions of new vinyl copolymers of linseed oil (1.0 professional man-year).

The Department also sponsors research conducted by foreign institutions under grants of PL 480 funds. Research on industrial chemical products involves grants to the Experiment Station for the Fats and Oils Industry, Milan, Italy, for studies on stereospecific polymerization of polyunsaturated fatty esters (2 years, 1965-1967), and to the Regional Research Laboratory, Hyderabad, India, for exploratory research on hydroxylation reactions of linseed and safflower oils (5 years, 1963-1968). During the reporting period research was completed on minor constituents of linseed oil and thermal polymerization of polyunsaturated oils at the Experiment Station for the Fats and Oils Industry, Milan, Italy, and on organometallic compounds in protective coatings at the Paint Research Station, Teddington, England. These research topics were under the headings chemical composition and physical properties, industrial chemical products, and protective coating products, respectively.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations did not report research in this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Minor constituents of linseed oil. At the Experiment Station for the Fats and Oils Industry, Milan, Italy, a C_{20} terpene alcohol, structurally similar to farnesol, has been isolated from linseed oil and identified as

geranyl-geraniol. This material had not been isolated from natural sources previously but had been postulated as an important intermediate in the biosynthesis of di- and tetraterpenes. Measurement of the effects of minor constituents on the spraying and wetting properties of linseed oil has proven to be very difficult because of the lack of a physical method sufficiently sensitive to show the small differences involved. Contact angle measurements appear to offer the most promise to date. Experimental work on this PL 480 project has been completed but the final report has not yet been received.

B. Industrial Chemical Products

1. Cyclic acids. All alkyl esters of C_{18} hydrogenated cyclic acids (HCal) that have been studied passed the oxidative stability requirement of specification MIL-L-7808 for jet lubricant base stocks. Further studies of a number of HCal diesters to determine their suitability as jet lubricants under the new and more rigorous specification MIL-L-23699 indicated that satisfactory viscosity index, pour point and low-temperature viscosity could be achieved by blending one of the diesters with a commercial low-viscosity oil (Herclube C). The mixtures did not, however, meet oxidative stability requirements when formulated with an available amine-type inhibitor.

Industrial reports indicate, however, that selection of an oxidation inhibitor and other components of the "additive package" required to impart satisfactory properties to a synthetic ester lubricant is a very critical and highly specific operation. It is well known that an "additive package" giving excellent results with one lubricant may be completely ineffective with another. In view of these facts, the results with HCal diesters are considered very encouraging, although it will probably be necessary to enlist the cooperation of an experienced industrial formulator of synthetic lubricants to develop an adequate "additive package."

In engineering research on hydrogenation of cyclic fatty acids, the use of adequate palladium catalyst (0.25 percent or higher) was found to be the most important factor in complete elimination (by reduction) of aromatic components that prevent achievement of maximum high temperature oxidative stability. Pilot-plant tests indicated that extractive distillation of monomeric fatty acids from the cyclization reaction could be a useful low-cost method for partial purification of cyclic acids. (In extractive distillation, methyl esters of polymeric fatty acids are added to the charge to be distilled.)

Chlorotrifluoroethylene and 1,1-dichloro-2,2-difluoroethylene reacted with a variety of C_{18} conjugated dienoic ester materials to give cyclo-addition products in yields as high as 80 percent. These reactions appear to be promising routes to products that should have unique properties.

2. Glyceride polymers. Under a PL 480 grant to the Experiment Station for the Fats and Oils Industry, Milan, Italy, studies on the ozonolysis of the dimer and trimer acids from heat polymerized linseed oil were continued.

Formaldehyde and propionaldehyde were the major products from the neutral fraction. Since these aldehydes were obtained in almost equal amounts, bond migration toward the terminal methyl group is indicated. Studies on the dibasic acids formed showed that essentially 50 percent of the double bonds nearest the carboxyl group remain in the original position. Experimental work on this project has been completed, but the final report has not yet been received. The grant is being replaced by a new one providing for studies on the stereo-specific polymerization of polyunsaturated fatty esters.

3. Hydroxylation of linseed oil. Two routes have been examined for introduction of single hydroxyl groups: sulfation followed by hydrolysis and epoxidation followed by reductive ring opening. The sulfation route was successful with methyl oleate and peanut oil, but not with polyunsaturated esters and oils. Formation of inner ethers may have interfered with the desired reaction. The epoxide route has not yet proved satisfactory because hydrogenation either failed to open the epoxide ring or yielded undesired products. This research is being conducted under a PL 480 grant to the Regional Research Laboratory, Hyderabad, India.

C. Protective Coating Products

1. Emulsion paints. Research on pigment interactions in water systems showed previously that attraction and flocculation of particles of two pigments occur at pH's between the isoelectric points of the pigments. Current results have revealed that particle size ratio of the interacting pigments determines the compositions of maximum and minimum mutual flocculation. Improved correction equations for vertical and horizontal interactions between particles have been developed that make possible greatly increased accuracy in the use of the Coulter counter. New equipment has been designed to permit precision control of variables in the study of factors affecting emulsification of linseed oil.

The new information on pigment interactions has provided an important advance in our understanding of the behavior of aqueous pigment suspensions. In particular, it has explained one of the more troublesome problems--unstable viscosity--encountered in development of acceptable linseed emulsion paints.

Three industrial companies are now marketing linseed oil emulsions for use in formulating paints. One of these companies alone is supplying some 150 paint manufacturers with linseed oil emulsion.

2. Linseed coatings for concrete. At Kansas State University, contract research on concrete specimens made with a sound quartz aggregate continues to indicate that air-entrained concrete can benefit from a linseed oil protective coating. Troweled surfaces were found to deteriorate more severely than formed surfaces, and reinforced beams more than plain beams. Application of a second coat after about a year of exposure would appear

advisable in practice to obtain best protection. Initial work at Kansas State on linseed oil coatings for simultaneous curing and protection of concrete is providing evidence that the surface "skin" of finished concrete is relatively weak and friable. Removal of this "skin" before applying linseed oil results in better protection to freezing and thawing. Unusual opportunities have arisen to test linseed emulsions under practical conditions of use for protection of concrete. With cooperation of the Chicago Park Board, special emulsions designed for low-temperature work were successfully applied at 36° F. to selected areas of the floor of the Grant Park underground garage. Arrangements have also been made for other practical tests of linseed emulsion concrete coatings in parking ramps and roads in Peoria, Illinois, and Washington, D. C.

3. New resins from linseed oil for use in water-soluble and other coatings. New types of potentially water-soluble vehicles were prepared by reacting various amounts of linseed alcohols with poly(maleic anhydride). A product containing 0.2 mole of linseed alcohols per maleic anhydride unit could be solubilized in water, but films were water-sensitive. Xylene solutions of a product containing 60 percent of linseed alcohols gave films having excellent air-drying properties and resistance to water, alkali and xylene. A resin comprising one mole of tris-(hydroxymethyl)aminomethane, two moles of linseed fatty acids and two moles of itaconic acid, solubilized as the dimethyl aminoethanol salt in aqueous isopropanol, gave rapid drying, water-resistant films. Films from a similar resin made with only one mole of linseed fatty acids dried slowly and lacked water resistance.

A series of new and promising film-forming resins was prepared by reaction of linseed dihydroxyamide with eight dibasic acids or anhydrides. Another type of polymer was prepared by condensation of 2-aminoethyl mercaptan with the half ester of maleic acid and linseed alcohols. This product gave tough but dark films when baked 15 minutes at 200° C. Linseed oil and hydrogen sulfide in methylene chloride solution were reacted at -69° C. by irradiation with ultraviolet light. Baked films from the treated oil (100° C.; 2 hrs.) had a hardness of 8 as contrasted with a hardness of only 2 for untreated linseed oil films baked under the same conditions.

These studies have resulted in availability of a variety of new products meriting further investigation. Those products that are sulfur derivatives are of particular interest because the mechanism by which their films dry or crosslink may result in formation of more stable bonds than are formed in drying of conventional products.

At North Dakota State University, reaction products of linseed monoaldehyde oil with maleic anhydride, polyvinyl alcohol or hydroxyethyl methacrylate gave films superior to those of linseed oil. Acetals of linseed monoaldehyde oil and polyols such as trimethylolpropane or pentaerythritol could be converted to urethane oils showing equal or better film properties than conventional urethane oils. In particular, these new urethane oils gave superior gloss and displayed greater ability to bridge the grain in heavily

grained wood such as mahogany. Because of their promising performance, it is planned to concentrate effort on these new coatings during the remainder of this contract research.

Initial studies on copolymers at Stanford Research Institute involved preparation of model triene compounds and preliminary copolymerizations of methyl linoleate or linolenate with ethyl acrylate, primarily to evaluate NMR for analysis of copolymers. In subsequent experiments, reactivity ratios for the copolymerization of ethyl acrylate or acrylonitrile with conjugated methyl linoleate were determined. The results indicated that it may be possible to prepare alternating copolymers.

4. Organometallic compounds in paints. Research under a PL 480 grant to the Paint Research Station, Teddington, England, has made considerable further progress, particularly in the development of gallate- and other polyhydric-phenol-modified coatings. The most promising vehicle, in terms of producing (without baking) a hard, adherent, anticorrosive coating on mild steel, was a gallate-modified, linseed-tung alkyd resin. Formation of these coatings involves direct complexing of phenolic groups in the vehicle with metallic iron in the substrate. "Soluble iron," particularly iron butoxide, was very effective in improving uniformity of the coatings. Adducts of vinyl phosphonic acid and conjugated fatty oils showed promise as antiflotation agents for certain pigments such as phthalo cyanine blue. Applications for additional public service patents have been filed to cover new developments during the past year. Laboratory work on this project has been completed, but the final report has not yet been received.

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AREA NO. 7: SOYBEANS
FOOD AND INDUSTRIAL USES FOR SOYBEAN OIL

Problem. Soybean oil is now the major edible oil of the United States and the most important source of nutritionally important linoleic acid. However, this oil contains an unstable component (linolenic acid) that limits its use as a liquid oil both domestically and in foreign markets. It is estimated that in 1964 about 4 billion pounds of soybean oil (about 90 percent of total domestic use) was consumed in edible products, of which about two-thirds was consumed in hydrogenated form as margarine and shortening. However, production of soybeans has increased rapidly, amounting to almost 700 million bushels in 1964.

The most promising outlets for oil from this ever-growing production of soybeans appear to be in foreign markets as edible oils and fats and in domestic industrial uses. The potential market for vegetable oils imported by Europe is estimated at over 7 billion pounds by 1966. For soybean oil to capture a growing share of this market, more information is needed to show how to eliminate unstable linolenic acid without loss of nutritive value, to determine the extent to which minor constituents influence flavor and other properties of the oil, and to discover methods for modifying hydrogenated soybean oil to achieve desired functional properties such as melting point and texture. This information would also serve as the basis for improving soybean oil for domestic use both as a liquid oil and in its hydrogenated forms. Some additional consumption in the United States might be anticipated because of extended utility resulting from these improvements, even though consumption of edible fats and oils mainly increases with population growth. To achieve the objective, a broad program of basic and applied research is required to provide more knowledge of the properties of linolenic acid and of minor constituents of soybean oil; of the changes that take place in these and other components during oxidation, hydrogenation, and heating; of the effects of these changes on flavor, nutritive value, stability, and other qualities of the oil; and of the effects of modification of glyceride structure on functional properties of hydrogenated forms of soybean oil.

As an industrial oil, soybean, like linseed oil, is faced with growing competition from synthetic products derived from nonagricultural sources. As an industrial source of linoleic acid, soybean fatty acids must also compete with tall oil fatty acids, a byproduct of paper manufacture. The best opportunity for increasing industrial applications of soybean oil appears, therefore, to be development of products that retain the glyceride structure of the oil. Thus, aldehyde oils, a recent discovery of Department scientists, appear to have a promising future, if current research and development is successful, in the multibillion-pound market for resins, fibers, coatings, plastics, plasticizers, pesticides, and paper and textile chemicals. To achieve the potential industrial value of aldehyde oils and

other soybean glyceride products, more fundamental information is needed on reactions of soybean oil that will preserve the glyceride structure and on the physical and chemical properties of the products. Upon this basis, development of a wide variety of new, industrially useful products should be possible.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing long-range program involving analytical, organic and physical chemists and chemical engineers engaged in basic and applied research on edible and industrial uses of soybean oil. A food technologist is also required by the program in connection with organoleptic evaluation of edible oils. Objectives of research on edible soybean oil are to identify undesirable flavor components of the oil, to develop basic information on the chemical changes and mechanisms involved in formation or suppression of these components and to apply the knowledge gained to the development of edible soybean oil having improved oxidative, thermal and organoleptic stability. Objectives of research on industrial utilization are to obtain new information on reactions of soybean oil and its components and to use this information to develop new or improved products for use by the chemical and other industries.

The Federal scientific effort for research on soybean oil totals 35.7 professional man-years. Of this number 7.1 are devoted to chemical composition and physical properties, 15.4 to edible utilization, and 13.2 to industrial utilization.

Research at Peoria, Illinois, on chemical composition and physical properties (7.1 professional man-years) is concerned with isolation and identification of components affecting flavor, heat, light, and storage stability of soybean oil and its hydrogenated products and with development of new and improved methods of separation and analysis for use in these studies. During the reporting period research was initiated with the specific objective of utilizing mass spectroscopy as a tool for solving difficult problems of analysis and characterization.

Research at Peoria, Illinois, on edible utilization of soybean oil (12.5 professional man-years) emphasizes basic and applied studies on selective hydrogenation as a means of stabilizing soybean oil by removal of linolenate. Research contracts (2.9 professional man-years) are in effect at IIT Research Institute, Chicago, Illinois, for development of heterogeneous selective hydrogenation catalysts (1.4 professional man-years); at Rutgers, The State University, New Brunswick, New Jersey, for basic studies on heterogeneous catalysts (1.0 professional man-year); and at the University of Illinois, Urbana, Illinois, for basic research on homogeneous catalysts (.5 professional man-year).

Research at Peoria, Illinois, on industrial utilization of soybean oil (9.0 professional man-years) involves exploratory studies to find new

reactions and products and basic and applied investigations of aldehyde oils and other aldehydic products. Research contracts (4.2 professional man-years) are in effect with Fabric Research Laboratories, Dedham, Massachusetts, for investigations on poly(ester-acetals) and poly(amide-acetals) derived from aldehyde oils (1.7 professional man-years) and with Archer Daniels Midland Company, Minneapolis, Minnesota, for pilot preparation of various aldehyde oil products needed for developmental investigations (2.5 professional man-years).

The Department also sponsors research on soybean oil conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to Gdansk Polytechnic, Gdansk, Poland, for studies on soybean sterols and their effect on stability of the oil (4 years, 1961-1965). During the reporting period research was completed on removal of trace metals from soybean oil with ion exchange resins at the Institute for Fats and Their Derivatives, Seville, Spain. Research on edible utilization is conducted under grants to the University of Granada, Granada, Spain, for studies on the effect of processing on frying quality of soybean oil (5 years, 1962-1967); Toyo University, Kawagoe, Saitama-ken, Japan, for research on hydrogenation of soybean oil (5 years, 1962-1967); Sugiyama Chemical Research Institute, Tokyo, Japan, for basic studies on the color reversion of soybean oil (2 years, 1964-1966); and Experiment Station for the Fats and Oils Industry, Milan, Italy, for studies on certain metal chelate compounds as catalysts for selective hydrogenation of soybean oil (2 years, 1965-1967). Research on industrial utilization involves grants to Queen Mary College, University of London, London, England, for basic studies on alkaline cleavage of polyunsaturated fatty acids (5 years, 1961-1966); and the Experiment Station for the Fats and Oils Industry, Milan, Italy, for research on oxidation with atmospheric oxygen to obtain new soybean oil derivatives (5 years, 1961-1966). During the reporting period research was completed on separation of mixtures of fatty acids at the University of Helsinki, Helsinki, Finland.

PROGRAM OF STATE EXPERIMENT STATIONS

Station research on food and industrial utilization of soybean oil involves study of the chemical, physical, and nutritional properties of the oil. Investigations directed to isolation, fractionation, and chemical identification of the compounds responsible for the reversion flavor of soybean oil continue. The mechanism concerned with flavor reversion is being studied. Content of non-carbonyl and carbonyl compounds in stable cottonseed oil is being compared with reverted soybean oil in the search for clues to explain the flavor reversion and to provide practical methods for preventing or retarding its formation.

Oilseed processing conditions and methods of extraction and recovery of oil from oil-bearing seeds are under investigation. Other research involves fundamental studies on the enzymatic formation of fats and oils in plants,

antioxidant mode of action, and the isolation of sulfur-containing lipids and glycolipids in plant tissues.

The total State effort devoted to soybean oil utilization is about 4.8 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Mass spectroscopy investigations. Studies have been undertaken to maximize the effectiveness of mass spectroscopic techniques for characterizing fatty acids and their chemical conversion products. Research on selective hydrogenation and other approaches to improvement of soybean oil as an edible oil, as well as research on converting soybean oil to new industrial products, present formidable problems in precise identification of products formed. By investigation of the explicit application of mass spectroscopy to these problems, the maximum capability of this powerful research tool should be realized.

In initial studies with model compounds, mass spectra were determined for methyl and ethyl sorbates, four methyl hexenoate isomers, ethyl 2- and ethyl 3-hexenoates, methyl 2-octenoates, and some of their deuterated derivatives in conjunction with mechanism studies of the catalytic hydrogenation of methyl sorbate in the presence of pentacyanocobaltate II ion. Mass spectrometric analysis of the hydrogenation product, methyl 2-hexenoate, using deuterium as a tracer, showed that the added hydrogen came from the water solution rather than from the gas phase. Three deuterium atoms were found rather than the expected two when the hydrogenation was performed in heavy water indicating that the third deuterium was added by exchange.

2. Components of autoxidized soybean oil. A new gas-solid chromatographic method of exceptional resolving power has been developed for analysis of volatile hydrocarbons found in autoxidized fats. Mixtures of C_1 to C_{10} paraffins and olefins are separated from each other, from acetylene, and from several cyclic hydrocarbons in less than 40 minutes.

This new technique has promise of providing a unique procedure for "fingerprinting" various vegetable oils and derived hydrogenated products. Hydrocarbon formation is among the first detectable signs of autoxidation; hence, the method may prove capable of providing an exceptionally sensitive indication of the previous history of a sample, as well as information pointing to its identity. Besides its value to research, the procedure obviously has potential for commercial use; e.g., for establishing specifications and verifying adherence to standards.

3. Removal of prooxidant metals. In studies under a PL 480 grant at the Institute for Fats and Oils and Their Derivatives, Seville, Spain, demetalization of soybean oil with macroreticular exchange resins was evaluated in

detail. Oils were passed through the resin columns, analyzed for metal content before and after treatment, and treated by the usual alkali-refining, bleaching and deodorization processes but with the addition of antioxidants and inactivators. Statistical analysis of the data indicates that the flavor stability was significantly improved by the procedures described. Laboratory work has been completed, but the final report has not yet been received.

4. Effects of sterols on flavor stability. Sterols of soybean oil were found to undergo highly complex transformations during alkali-refining and bleaching. Alkali and oxygen adsorbed in the bleaching earths play an important role in inducing these changes. Bleaching appeared to effectively remove apolar steroids from the oil. This research is being conducted under a PL 480 grant at Gdansk Polytechnic, Gdansk, Poland.

B. Edible Utilization

1. Selective hydrogenation. Studies on homogeneous catalytic hydrogenation with pentacyanocobaltate II showed that hydrogenation occurred only when the substrate could coordinate with the catalyst. Thus, only higher fatty acids containing triple conjugation and a trans bond could be hydrogenated.

Mixtures of copper and chromium salts reduced with sodium borohydride yielded extremely selective (selectivity ratio = 7) catalysts in small-scale (1 gm.) hydrogenations of soybean oil. Attempts to use these catalysts for hydrogenation of 2-liter samples of oil have so far provided selectivity ratios of only 2.5. In the presence of dimethyl formamide (DMF) 81-percent yields of monoene with concomitant 13.4-percent yields of stearate were obtained with palladium-carbon catalysts as compared to 45 percent and 35.7 percent, respectively, without DMF. The product prepared in DMF, when ozonized, yielded 73 percent of C_9 and C_{12} dibasic acids compared to 46 percent without DMF. This observation shows that there was less wandering of the double bonds when DMF was present.

Nickel (III), cobalt (III), copper (II) and iron (III) acetylacetonates, when used as catalysts for hydrogenation of soybean and linseed methyl esters, showed high selectivity toward linolenate (selectivity ratios of 3.5-4.1). These reactions were conducted in methanol solution. However, attempts to hydrogenate soybean oil with nickel (III) acetylacetonate under these conditions resulted in conversion of the triglycerides to methyl esters. Alcoholysis was avoided by changing the solvent but the catalyst was at best only slightly active under these conditions.

Iron pentacarbonyl was shown to be an effective conjugation reagent, making possible the production of oils in which 93 to 97 percent of the poly-unsaturated acids were conjugated, mainly in the all-trans-configuration. The method has been applied to soybean, linseed and safflower oils.

During investigation of extractive rearrangement of soybean oil, it was discovered that certain solvents enhance the selectivity of hydrogenation catalysts. Thus, when a mixture of linseed and safflower oils was hydrogenated in dimethyl formamide, the selectivity ratio for the catalyst increased from 1.8 to 4. Other solvents that behave similarly include furfural, acetonitrile, tetramethyl urea and trimethyl phosphate. By use of an analog computer it can be shown that when the selectivity ratio is of the order of 4.0 it is possible to hydrogenate soybean oil selectivity to less than 2 percent linolenic acid without necessitating winterization to remove excessively hardened oil. The results achieved during the year show that selectivity ratios of 4 or more can be obtained by laboratory procedures. Considerable further study will be needed to determine if industrially feasible processes of equivalent effectiveness can be developed.

In the contract research program, scientists at the University of Illinois discovered that stannous chloride markedly changes the selectivity of hydrogenations catalyzed by platinum-triphenyl phosphine complexes. Thus, soybean esters could be selectively hydrogenated to trans monoenes without forming saturates. Several new palladium complexes also proved to be highly effective catalysts.

At IIT Research Institute comparative experiments with Linde Type X molecular sieves impregnated with nickel and with rhodium showed no consistent preference for attack at the 15,16 bond of linolenate. The rhodium catalysts produced much more trans bonds than did the nickel catalysts. The nickel catalysts made with X-type sieves were 10 to 20 times more active than similar catalysts made with A-type sieves. In the research at Rutgers, the principal achievements were improvement of resolution of infrared emission spectra for oleic acid and development of an effective stirring device for microhydrogenation.

2. Hydrogenated-winterized soybean oil. Study of acetone-oil mixtures used in preparation of hydrogenated-winterized soybean oil showed that phase separations could be easily achieved by adjustment of temperature or by addition of 3 to 8 percent of water. In producing hydrogenated-winterized soybean oils, proper use of phase separation techniques could eliminate distillation costs, minimize solvent losses and provide a basis for development of a continuous solvent winterization process.

3. Frying quality of soybean oil. In studies under a PL 480 grant at the University of Granada, Granada, Spain, tests conducted in a professional school for country people who were habitual consumers of olive oil showed they were unable to identify whether olive oil or soybean oil had been used in the preparation of their usual meals. These results were the same as those found when two university groups (men and women) were tested.

4. Partial hydrogenation of soybean oil. Studies with copper-nickel catalysts have progressed to preparations of approximately 4 kg. of hydrogenated-winterized soybean oil with linolenate contents of 5 to 6 percent.

Stability was apparently improved when metallic impurities that result from the use of copper-nickel catalyst were inactivated. Reuse of the catalyst showed appreciable loss in activity after five repeated runs, whereas nickel catalyst in the United States is frequently used much longer. Longevity of catalyst activity appeared to be associated with nickel content. A 9.5:1:10 (copper:nickel:kieselguhr) catalyst had a longer life than a 9.5:0.5:10 catalyst. This research is being conducted under a PL 480 grant at Toyo University, Kawagoe, Saitama-ken, Japan.

C. Industrial Utilization

1. Oxidative cleavage of soybean oil and its fatty acids. Further study of solvent systems for ozonization showed that nearly quantitative conversion of double bond to aldehydic products was achieved with mixtures of acetic or propionic acid and ethanol, n-propanol or isopropanol. A 1-butanol-propionic acid system (aldehyde yields over 90 percent of theory from pure methyl oleate) appeared promising for industrial use. As another approach, soybean oil methyl esters were mechanically emulsified in water and ozonized. Subsequent yields of aldehyde were 86 percent of theory by chemical reduction or 81 percent by hydrogenation. An economic analysis is now needed to decide between inexpensive aqueous systems, which give lower yields of product, and the more expensive organic solvents, which give high yields. It is anticipated that this type of evaluation will be made under the recently initiated contract project at Archer Daniels Midland Company, which provides for developmental studies on aldehyde oils.

2. Aldehyde oil derivatives. By use of optimal time, temperature and pressure and of a nonpolar solvent to repress amonolysis of the ester group, methyl and butyl azelaaldehydates have been converted to the 9-amino esters in yields of 92 and 93 percent, respectively. Solvents such as heptane and decalin were found to be nearly as satisfactory as methyl cyclohexane for use in reductive amination of methyl azelaaldehydate (MAZ). The C_3 analog of the MAZ pentaerythritol acetate was synthesized and polyesterified with ethylene glycol. The polymer was lower melting and less soluble in common solvents than the corresponding MAZ poly(ester-acetal).

At Fabric Research Laboratories, poly(ester-acetals) were prepared from the glycerol (I) and 2-hydroxymethyl-2-methyl-1,3-propanediol (II) acetals of MAZ. A study of esterification catalysts showed that lead acetate trihydrate effectively promoted, at temperatures below 250° C., ester interchange in the polymer from I without causing acetal interchange. The polymer from II crosslinked under these conditions.

3. Separation of fatty acids. In final phases of research under a PL 480 grant to the University of Helsinki, Helsinki, Finland, crystallization methods were developed to separate polyunsaturated fatty-acid concentrates from soybean and linseed fatty acids in over 90 percent purity and in satisfactory yields. Liquid-liquid extraction of the soybean concentrate gave linoleic acid in over 91 percent purity, and linolenic acid in over

98 percent purity was obtained from the linseed concentrate. The combined crystallization-extraction process should be an improvement over liquid-liquid extraction for economical production of high purity linoleic and linolenic acids.

4. New derivatives. Basic information on the reactions that occur when fatty acids are fused with alkali has been obtained with reactions involving potassium deuteroxide. Deuterium replaced hydrogen on carbons 2 through 5 but did not replace hydrogen on carbons 12, 13 or 14 in myristic acid. Myristic acid gave small amounts of lauric acid, and it is presumed this reaction occurred by dehydrogenation followed by the Varrentrapp reaction. Studies with saturated and aromatic acids indicated that little if any attack in this reaction occurred at carbon 3. Alkali fusion with sodamide gave high yields of saturated hydrocarbons of one less carbon atom. Substitution in the 2 and 4 positions stabilized the saturated acids to alkali fusion but substitution in the 3 position was much less effective. These studies are in progress under a PL 480 grant to Queen Mary College, University of London, London, England.

At the Experiment Station for the Fats and Oils Industry, Milan, Italy, research under a PL 480 grant has been directed toward testing various metal chelates with a variety of ligands for their effect on rate of autoxidation of methyl oleate. This work has involved the preparation and purification of metal chelates of individual fatty acids. Not only has the rate of catalytic autoxidation been studied but information has also been gained on the decomposition of the formed hydroperoxides. The data are being used to correlate stability constants of chelates with their infrared structures, with the view of developing theories to explain the differences in catalytic activity observed.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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AREA NO. 8: SOYBEANS
FEED, FOOD AND INDUSTRIAL USES FOR MEAL AND PROTEIN

Problem. Production of soybeans has increased rapidly to almost 700 million bushels in 1964. For profitable disposition, now and in the future, of the growing supplies of meal from U. S. soybeans, improved feed products and new food and industrial uses are needed. Europe is developing a mixed-feed industry that needs high-protein concentrates. This market could approach that in the U. S. which uses high-protein meal from 400 million bushels of soybeans. For U. S. soybeans to achieve the maximum share of this market, more fundamental information is needed on the proteins and other nutritionally important constituents of soybeans and on the effects of processing on these components. Such information should make possible the production of feeds from soybeans having maximum feeding value that would meet the requirements of foreign markets as well as help maintain or increase the use of soybean feeds in the U. S.

U. S. soybeans could play a dominant role in alleviating the world shortage of dietary protein if more information were available on utilizing soybeans and soybean meal, flour, protein and protein concentrates in food products tailored to meet the nutritional and palatability requirements of foreign markets. That the possibilities are very real for increased utilization of soybeans in foreign food is indicated by recent work of the Department that showed how to use U. S. soybeans for Japanese foods. The result of this work was that a market for selected soybeans for Japan was opened that now exceeds one million bushels per year. If U. S. soybeans are to achieve the maximum share of foreign food markets, basic information on nutritionally important components and effects of processing on these components will be needed. In addition, better knowledge will be required of how to use soybean protein products in foodstuffs that will be acceptable abroad.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing long-range program involving organic and physical chemists and biochemists engaged in basic research on the characterization of components of soybean meal and protein and application of the knowledge gained to solution of problems encountered in processing and utilization of soybean meal and protein.

The Federal scientific effort on utilization of soybeans and soybean meal and protein totals 14.5 professional man-years. Of this number 7.1 are devoted to chemical composition and physical properties and 7.4 to food products.

Research at Peoria, Illinois, on chemical composition and physical properties (7.1 professional man-years) involves basic studies on isolation and

characterization of components of whey proteins and on heat gelation of alcohol-washed protein.

Research at Peoria, Illinois, on food products (7.0 professional man-years) is devoted to development of information on specially processed soybean products pertinent to their use in high-protein foods for foreign markets. One segment of this research--engineering studies on conversion of soybeans to high-quality, full-fat flours--is supported by the Agency for International Development. A research contract (.4 professional man-year) at the University of Illinois, Urbana, Illinois, is concerned with investigation of factors possibly present in soybeans that could cause digestive disturbances.

The current program at Peoria, Illinois, does not include research specifically directed to industrial or feed products.

The Department also sponsors research on utilization of soybeans conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to the University of Edinburgh, Edinburgh, Scotland, for investigations on polysaccharides of soybeans (5 years, 1961-1966); to the Weizmann Institute of Science, Rehovot, Israel, for research on complexes between soybean protein and other components of the meal (5 years, 1961-1966); to Kagawa University, Takamatsu, Japan, for a chromatographic study of soybean sugars and oligosaccharides (3 years, 1963-1966); and to the University of Tokyo, Tokyo, Japan, for studies on soybean sterols in defatted meal (4 years, 1963-1967).

Research on food products involves grants to the Central Miso Institute, Tokyo, Japan, for studies on miso made from dehulled soybean grits (4 years, 1962-1966); Bar-Ilan University, Ramat Gan, Israel, for studies on miso-type food products for use in Israel (3 years, 1962-1965); Israel Institute of Technology, Haifa, Israel, for evaluation of the quality of isolated soybean protein for use in Israeli foods (4 years, 1962-1966); Japan Tofu Association, Tokyo, Japan, for studies on the use of U. S. soybeans for making tofu (4 years, 1963-1967); Institute of Chemistry, Academia Sinica, Taipei, Taiwan, for investigation on preparing Chinese cheese from soybeans (5 years, 1963-1968); Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan, for studies on improved strains of Saccharomyces rouxii for making shoyu and miso (5 years, 1963-1968); and University of Tokyo, Tokyo, Japan, for investigations on the flavor components of enzymatically or chemically modified soybean meal and proteins (3 years, 1964-1967).

Research on feed products involves a grant to the Hebrew University, Rehovot, Israel, for basic studies on soybean saponins (5 years, 1961-1966).

PROGRAM OF STATE EXPERIMENT STATIONS

The current station program involves both basic and applied research on soybean protein and meal utilization. Much of the basic research is aimed

at characterization of soybean meal and protein. Application of this information to utilization is also made through several studies involving feed use of the meal. Basic studies seek to characterize the proteins and identify such biologically active components as proteolytic enzymes and their inhibitors. Other work is being directed to separation and identification of the proteins in soybean whey. Genetic effects as expressed in different varieties are being observed. Peptide structure of the individual purified proteins is investigated. Other researches involve study of the basic mechanisms of the biosynthesis of proteins.

In the area of food use, production of high protein fermented foods such as tempeh from soybeans is the subject of a pilot study. This involves methods of processing, fractionating or modifying soybeans to produce low-cost, protein-rich foods of value for feeding infants and children.

Extensive economic feasibility studies are in progress. These range from use of meals in livestock feeds to the impact of the common market.

Total research effort devoted to soybean meal utilization is 5.8 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Basic studies on soybean protein. Insolubility of isoelectrically precipitated protein has been shown to be caused in part by disulfide bonding affecting the 7S and 11S components. The 2S and 7S fractions also are involved in formation of insoluble material apparently by a mechanism not involving disulfide bonding, since mercaptoethanol did not solubilize such material. Analysis of a number of soybean protein fractions for their carbohydrate content revealed that the proportion of carbohydrate in 7S protein is 17 times as great as in 11S protein. The present results provide additional evidence that the 11S and 7S fractions are not merely aggregates or polymers of identical subunits.

Gel electrophoresis has proven very effective for characterization of soybean proteins. Whey proteins were resolved into 18 bands. The four trypsin inhibitors isolated at the Northern Division and reported last year were found to be heterogeneous; for example, inhibitor A_2 showed six or more minor bands.

Other studies showed that trypsin inhibitors account for 30 to 50 percent of the growth inhibitory activity of raw meal and for nearly all of the pancreatic hypertrophic effect. The trypsin-trypsin inhibitor reaction was shown to involve cleavage of a sensitive bond in the inhibitor. These findings provide basic information important in development of soybean foods and feeds having optimum nutritive value.

2. Soybean sugars, oligosaccharides and polysaccharides. Under a PL 480 grant at the University of Edinburgh, Edinburgh, Scotland, studies showed that hydrolysis of soybean cotyledon polysaccharides gave the following monosaccharides: galactose, galacturonic acid, arabinose, xylose, fucose, rhamnose, glucose, mannose, and aldobiouronic acid. The results indicate that these polysaccharides are a mixture of a neutral arabinogalactan and an acidic pectin-type complex, with structural features similar to gum tragacanth. The acidic polysaccharide fraction is the major one.

In other PL 480 research at Kagawa University, Takamatsu, Japan, quantitative analyses of the sugars in U. S. and Japanese soybean varieties appear near completion. Preliminary studies have shown an increase in reducing sugars when soybean meal is autoclaved. However, the amounts of some individual sugars increased, whereas decreases were observed for others.

3. Complexes of soybean protein with other meal constituents. Scientists at the Weizmann Institute of Science, Rehovot, Israel, have isolated a glycopeptide from soybean hemagglutinin, thereby establishing the glycoprotein nature of this protein. This work is being conducted under a PL 480 grant.

4. Soybean sterols. Under a PL 480 grant at the University of Tokyo, Tokyo, Japan, scientists developed a method for extracting the sterols from soybeans and for analyzing them on a chromatographic column. Application of this method has revealed the presence of an unknown sterol fraction tentatively identified as an esterified sterol glycoside.

B. Food Products

1. Flavor and nutritive value of soybean food products. An intensely bitter fraction was isolated from soybeans. Nine components were found in this fraction, of which a phenolic acid (ferulic acid) and two isoflavone aglycones (genistein and daidzein) were identified. Saponins crystallized from alcohol extracts of soybeans did not have a bitter taste. Presence of a leucoanthocyanin in soybean meal was confirmed. The indication that bitterness in soybeans is not caused by saponins but may instead be due to phenolic acids, flavenoids and/or unidentified components is an interesting lead that represents a departure from previous concepts. In peanuts, for example, bitterness has been attributed to saponins.

Studies at the University of Illinois showed that low-molecular-weight constituents of soybean whey solids and of the 80-percent ethanol extractives of soybean meal are the primary cause of flatus in humans and dogs. Sodium proteinate and caseinate completely inhibited the flatus activity of whey solids or meal extractives. Commercial food-grade preparations of isolated soybean protein did not produce flatus.

In vivo and in vitro studies show that flatus is produced via activity of intestinal microflora following ingestion of flatus-causing foods. Isolated

cultures of the microflora of the colon of dogs are now being used in an assay for the flatus factor.

These results suggest that a useful solution to the flatus problem may soon be achieved; i.e., addition of sodium proteinate or caseinate to the food. Further work will be needed to confirm the present findings and to evaluate the practical value of this solution to the problem. Development of a rapid in vitro assay for flatus should greatly accelerate successful conclusion of this work.

2. Full-fat soybean flour. The Agency for International Development has authorized support of a project covering engineering research on production of full-fat soybean flour. This research continues and expands studies begun under the UNICEF Cooperative Program. Preliminary studies under this new project have been directed toward investigation of atmospheric steaming, water-immersion cooking and dry heating or roasting as possible simple methods for producing soy flour in countries having minimum facilities. The initial moisture content was shown to be a critical factor in achieving very rapid cooking with minimum protein denaturation. The pediatric evaluation of extruder-cooked full-fat soybean flour, conducted by UNICEF in Taiwan, is nearly complete. Results are very favorable and almost identical to those with cow's milk.

3. Studies on miso and shoyu. These studies are being conducted by several foreign institutions under PL 480 grants.

Results of large-scale miso fermentations at the Central Miso Institute, Tokyo, Japan, showed that the number of yeasts is much greater in fermentation of soybean grits than in fermentation of whole soybeans and that miso from grits scored higher in organoleptic tests than did miso made by the traditional method. At Bar-Ilan University, Ramat Gan, Israel, defatted soybean flakes were used successfully to produce a Japanese-type miso. Use of commercial enzymes enabled shortened fermentations. Rice koji (used as a "starter") could be replaced with koji made from corn and other cereals.

In the search for better strains of the organism Saccharomyces rouxii for use in making miso and shoyu (soy sauce), a large number of strains have been evaluated in terms of salt tolerance, flavor, alcohol formation and growth in a high nitrogen medium. Endeavors to find a key compound that could be used to characterize the total flavor in shoyu were unsuccessful. This research is being conducted by the Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan.

4. Quality of isolated protein for use in Israeli-type foods. In studies conducted under a PL 480 grant at the Israel Institute of Technology, Haifa, Israel, investigation of several processing variables resulted in improved yields, color, taste, nutritive value, and functional properties of isolated soybean protein. The pilot-plant data obtained are of

particular significance to soybean protein technology as a basis for improving and expanding the use of soybeans for food in developing countries.

5. Chinese cheese (sufu). Studies have now established that Actinomucor is the fungus used in the commercial production of sufu in Hong Kong and Taiwan. However, in preparation of sufu in the home a variety of species of Mucor are involved. Both Actinomucor and Mucor species were found to produce proteinase and alkaline phosphatase. In addition, certain isolates had active lipases. This research is being conducted under a PL 480 grant by the Institute of Chemistry, Academia Sinica, Taipei, Taiwan.

C. Feed Products

1. Effects of saponins on nutritional quality of soybean feeds and foods. In research under a PL 480 grant at Hebrew University, Rehovot, Israel, a new saponin has been isolated from soybeans and characterized. Its aglycone has been designated soysapogenol E. Soysapogenols A, B, C, and D were isolated some 15 years ago by Swiss workers. The new soysapogenol is a triterpene alcohol similar to soysapogenol B, differing only in that it has a hydroxyl group at C_{21}.

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*Research supported by PL 480 funds.

AREA NO. 9: REPLACEMENT CROPS UTILIZATION POTENTIAL - NORTHERN REGION

Problem. Farmers could achieve more economic use of their land if new and profitable crops were available for their choice that would have different end-use patterns from those presently grown. For example, it would be advantageous to develop a new oilseed crop yielding unique fatty acids that could find industrial use in applications for which acids from presently available domestic oilseed crops are unsuitable. To develop a new crop, three basic steps are involved: (1) survey of wild plants, in cooperation with plant scientists, to identify those having both potentially valuable components and promising agronomic potential for use in the United States; (2) detailed physical and chemical characterization of components of interest to obtain clues to likely end uses; (3) selection of the most promising species followed by additional utilization research to explore uses and demonstrate industrial potential and by additional agronomic research to establish proper cultural practices and to select the best strains and varieties. Only after these steps have been successfully accomplished can a proposed new crop be offered to agriculture and industry for introduction and development. Obviously, a program of this type is a long-range one. Yet, whether the future of agriculture involves conditions of surplus, of greater emphasis on foods and feeds, or of necessity for greater national self-sufficiency, the nation will benefit from availability of optimum, practical crop plants to serve its needs.

To achieve the objective, survey and characterization work needs to be greatly increased, since the greater the number of species examined, the greater will be the opportunities for finding plants meeting the criteria of high utilization and agronomic potentials. Work of the Department has already revealed several promising sources of new potentially valuable water-soluble gums, pulp fibers, and oils containing unique fatty acids such as hydroxy-unsaturated acids, capric acid, epoxidized acids, and unusual long-chain fatty acids. In order to demonstrate the potential of these new materials, further work is required on their physical and chemical properties and reactions, on processing to obtain maximum recovery from source plants, and on byproducts from processing, such as oilseed meals.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a long-range continuing program of research involving analytical and organic chemists and chemical engineers engaged in examination of uncultivated plants to find unusual and potentially useful components and in detailed characterization and evaluation studies of selected components that have the greatest industrial potential and that are obtainable from agronomically promising plants. Plants or seeds for this program are obtained by cooperation with Crops Research Division which procures material from domestic and foreign sources by means of

collecting trips or from experimental plantings. Materials from abroad are also made available through Crops Research Division PL 480 projects providing for collecting activities by foreign investigators. All seeds and plants are submitted to a broad chemical screening program to identify sources of unusual and potentially useful components such as oils, fibers, gums, amino acids and proteins. Components of interest from plants rated by Crops Research Division as having a reasonable agronomic potential for the United States are characterized to obtain clues to areas of utilization of probable interest to industry. On the basis of the results, plants having the highest agronomic potential and containing components of greatest potential industrial value are selected for more intensive utilization research. This utilization research is divided among the four Utilization Research and Development Divisions.

The Federal scientific effort devoted to research on replacement crops at Peoria, Illinois, totals 27.1 professional man-years. Of this number 16.0 are concerned with chemical composition and physical properties; 8.7 with industrial utilization of new oilseeds; and 2.4 with industrial utilization of new gum and fiber plants.

Research at Peoria, Illinois, on chemical composition and physical properties (16.0 professional man-years) involves conduct of the program on screening uncultivated plants for unusual and potentially useful oils, fibers, gums, amino acids and other components; organic chemical characterization of selected fractions and components, especially new oils and fatty acids; and studies on properties of new plant fibers.

Research at Peoria, Illinois, on industrial utilization of new oilseeds (7.1 professional man-years) involves studies on processing of erucic acid oilseeds to obtain oil and meal and investigations on utilization of erucic acid and its derivatives. A research contract (1.6 professional man-years) is in effect with Southern Research Institute, Birmingham, Alabama, for studies on preparation and evaluation of polyamide resins derived from crambe oil.

Research at Peoria, Illinois, on industrial utilization of new gum and fiber plants (2.4 professional man-years) is concerned with development of methods for recovery of gums from plants; with evaluation of plant gums in industrial applications; and with studies on pulping new fiber plants and evaluation of the pulp in paper, structural boards and related products.

The Department also sponsors research in this area conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to the Institute of General Chemistry, Warsaw, Poland, for determination of glyceride structure of erucic acid oils (5 years, 1962-1967); and to the Swedish Seed Association, Svalof, Sweden, to find new erucic acid oilseeds (5 years, 1963-1968).

PROGRAM OF STATE EXPERIMENT STATIONS

Discovery and preservation of valuable plant germ plasm is a continuing objective of the station program in new crops. Much of the research in this area is being done via four regional projects and in cooperation with regional centers. A large portion of the work is cooperative with USDA. Each year many plant introductions are grown and evaluated. Annual and perennial crops possessing potential for industrial or agricultural use are further evaluated for agronomic and chemical qualities. These include crops for paper pulp, pigments, drugs, tannins, essential oils, insecticides, polysaccharide gums, and oils rich in acids of unusual structure. Assay of native and introduced tropical plants for products of economic value receives special attention. New varieties of fruits, vegetables, and grasses better resistant to disease and drought are continually sought.

Basic aspects of this program involve study of the biochemical and physiological basis for differences in crop plants. Attempts are made to determine if differences in biochemical or physiological processes can be associated with particular factors related to quality. Information concerning carbohydrate transformations is sought through study of carbohydrate formation and enzyme mechanisms. Horticultural specialty crops are gaining in importance. A number of studies are underway to facilitate rapid development of this industry.

The total scientific effort devoted to replacement crops is 8.4 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Screening for new industrial oils. Since the last report, screening analyses were performed on 822 seed samples and 258 samples of oil were analyzed. Oils from four new species found to contain 12,13-epoxy-cis-9-octadecenoic (vernolic acid) were: Crepis vesicaria and C. kitaibelii (family Compositae); Cephalaria joppica (Dipscacaceae); and Valerianella radiata (Valerianaceae). The seed oils from these species contained 50, 53, 33, and 31 percent of vernolic acid, respectively. An additional Crepis (C. thomsonii) with high content (60 percent) of crepenynic acid was found. Impatiens edgeworthii contained 61 percent of the conjugated tetraenoic acid, parinaric acid. The highest concentration reported previously was 56 percent. Crambe seed selections from Texas AES showed oil content of 39.5-47.0 percent and erucic acid content of 55.4-58.7 percent. Seed oil of Acanthosyris spinescens contained 34 percent of normal C_{17} acids. These studies provide essential guidance to agronomists in selection of species for experimental planting and to utilization research scientists in selection of the most promising oils for further study and evaluation.

Under a PL 480 grant to the Swedish Seed Association, Svalof, Sweden, scientists have measured erucic acid content of a large number of samples of seed oils and have determined amounts and types of thioglucosides in the seeds. Genetic variability in erucic acid content of crambe oil was found to be much less than that of Brassica plant seeds but still sufficient to offer encouragement that higher erucic strains of crambe can be developed. High erucic lines of white mustard and of rapeseed have been selected that contain just over 60 percent erucic acid. These will be used in attempts to achieve still further increases in erucic acid content.

2. Characterization of new seed oils and components. Laballenic acid, which comprises 16 percent of the fatty acids of Leonotis nepaetifolia seed oil has been fully characterized as 5,6-octadecadienoic acid. Preliminary characterization of the conjugated tetraenoic acid from the seed oil of Impatiens edgeworthii indicates that it is cis-9,trans-11,trans-13,cis-15-octadecatetraenoic acid. Nine acetylenic fatty acids were found in the seed oil of Acanthosyris spinescens. Seven of the nine are new. One component acid of the seed oil of Acanthosyris spinescens has been characterized as the previously unknown 7-hydroxy-trans-10,16-heptadecadien-8-ynoic acid.

At the Institute of General Chemistry, Warsaw, Poland, under a PL 480 grant, hydrolysis with pancreatic lipase was applied to crambe oil and to fractions separated from it by column chromatography on alumina. Results confirmed earlier work in demonstrating that erucic acid is nearly all esterified to the primary alcohol groups of glycerol (α-position). However, one crambe oil fraction, representing about 12 mole percent of the oil, had almost 25 percent of the erucic acid in the β-position.

3. Characterization of the components of crambe. Either (R)-goitrin or nitrile (1-cyano-2-hydroxy-3-butene) and related products, or both, could be produced in enzymatic hydrolysis of thioglucosides in crambe meal, depending upon pH, temperature, amount of water and previous treatments of the meal. Increased formation of (R)-goitrin and decreased formation of nitrile and related products were observed during endogenous enzyme hydrolysis of crambe meal that was obtained from aged seed, subjected to high storage temperatures, or subjected to a dry heat treatment. The crystalline polypeptide crambin is, like crambe myrosinase, insoluble in water, but it is not the enzyme per se. In enzymatic hydrolysis the presence of 2-mercaptoethanol favored nitrile formation. Analysis of 16 new accessions of crambe for total thioglucosides showed no significant variation. Sinapine was identified as the major cationic fluorescent substance in crambe meal extracts. Enzymatic hydrolysis of thioglucosides in the meal did not change the sinapine content. Sinapine from crambe was identical to that from rape and could be completely removed by extraction of crambe meal with hot methanol. Information is being steadily accumulated on which an effective process for producing palatable nutritious crambe meal can be based. The present results extend our knowledge of the complex chemistry of crambe enzymes and of thioglucoside conversion and provide more of the detailed background needed for controlling processing and product

quality. The possible relationship of sinapine, a bitter-tasting substance, to palatability of crambe meal is an interesting lead that may prove highly significant.

4. Screening for new seed mucilages. Dry-milled gum from Cassia occidentalis, when evaluated as a wet-end additive for paper, performed as well as industrial grade guar gum in all respects but brightness. Research on mucilages has been maintained at a minimum level during the year because of assignment of personnel to higher priority research, such as that on crambe.

5. Screening for new pulp fiber plants. Continued screening of sorghums for pulping potential revealed six lines meriting further study. In fact, several lines appeared to have exceptional promise as fiber crops. However, additional data on crop yields and physical structure are needed to evaluate the economics of sorghum for this purpose.

Of a number of new plant samples under evaluation as fiber sources, Crotalaria eriocarpa exhibited favorable potential.

B. Industrial Utilization of New Oilseeds

1. Studies on utilization of crambe meal. A 196-day beef cattle-feeding trial of crambe meal from the commercial-scale run reported last year was conducted at the University of Nebraska. Initially, poor palatability resulted in poor consumption. With the aid of molasses and pelleting, good feed intake and efficiency were obtained with 33 percent crambe meal in the protein supplement. At levels of 66 percent or 100 percent, poorer palatability and smaller gains were noted. However, at all levels feed efficiencies and carcass grades were comparable to those obtained with soybean meal. No thyrotoxicity or other abnormalities of organs were found in crambe-fed animals.

Ammonia treatment of crambe meal appeared to eliminate toxicity when fed to chicks in a 4-week test at the 20-percent level. University of Nebraska reported improved acceptability of the ammonia-treated meal to cattle. Analytical tests showed absence of thiooxazolidone and sinapine.

In feeding studies with rats, full-fat crambe meal enzymatically converted at low moisture levels and extracted with aqueous acetone supported growth at 90 percent of the growth rate of control animals. When fed at 28 percent of the diet, no palatability problem was evident. Similar treatment of defatted meal gave a product that supported rat growth at 88 percent of that of the controls over a 90-day period. Autopsies revealed no abnormalities of organs.

2. Studies on utilization of erucic acid. Adducts of brassylic acid with from 1 to 100 moles of ethylene oxide have now been synthesized. The products cover the whole range of oil/water and water/oil surfactants and

are comparable in surface activity to analogous commercial products. Oxidative ozonization of a water emulsion of erucic acid dissolved in propionic acid was successfully effected by continuous processing in the pilot plant of an industrial concern. The product (185 pounds) was high purity (>95 percent) brassylic acid. Brassylate esters of mixed alcohols were superior to the dicyclohexyl ester as low-temperature plasticizers for polyvinyl chloride (PVC) in tests conducted at the Eastern Division. Vinyl 2-methylpentyl brassylate was prepared for tests as an internal plasticizer for PVC.

At Southern Research Institute, 13-aminotridecanoic acid was prepared and converted in small-scale trials to nylon-13. 1,13-Dicyanoundecane has also been successfully prepared, but reduction to diamine revealed difficulties requiring further study. In a comparison of nitric acid oxidation and ozonization for cleavage of erucic to brassylic acid, nitric acid oxidation gave a higher yield but was difficult to control. Also, special treatments were necessary to remove nitrogenous impurities from the product.

A memorandum of understanding was negotiated with McClouth Steel Company for tests of crambe oil as a lubricant in continuous casting of steel, a potential market of 6 to 8 million pounds per year. This study, which consumed oil at the rate of 2,500 pounds per month, showed that crambe oil performed exceptionally well in this application. In fact, if an adequate supply of oil were available, large amounts could readily be marketed for this purpose. Interest in acquiring quantities of crambe oil for still other industrial end uses has been expressed by several companies. It is evident, therefore, that prospects for industrial utilization of crambe oil are excellent, and that interest continues to grow. It is noteworthy that the supply of oil is proving to be the limiting factor.

C. Industrial Utilization of New Fiber Plants

1. Kenaf for pulp and paper. Kenaf harvested green and stored in chopped form appeared to be superior to "ensiled" green kenaf or post-frost harvested kenafs as a raw material for bleachable sulfate pulps. Dry storage appeared superior to wet storage of green kenaf for sulfate pulping. A newsprint-type paper made from up to 90 percent of chemi-mechanical pulp from unfractionated kenaf had insufficient opacity although other properties exceeded published specifications for several commercial newsprints. Unfractionated kenaf pulps were successfully used to formulate furnishes meeting the requirements for magazine and bond papers. The experimental papers had properties equal to or exceeding commercial requirements. The growing industrial interest in kenaf is evidenced by negotiation of a short-term memorandum of understanding with Hudson Pulp and Paper Corporation, Palatka, Florida, covering cooperation in kenaf storage studies. At least two companies will have experimental plantings this year. Many inquiries from paper companies have been received. Much of the current interest was generated by the successful planting at Florida AES, for which dry matter yields of 10 to 15 tons per acre were reported.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

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Bagby, M. O., Smith, C. R., Jr., and Wolff, I. A. 1964. A naturally occurring allenic acid from Leonotis nepetaefolia seed oil. Chem. Ind. (London) (45), pp. 1861-1862.

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Mikolajczak, K. L., and Bagby, M. O. 1965. Partial reduction of α-eleostearic acid with hydrazine. J. Am. Oil Chemists' Soc. 42(1), pp. 43-45.

Mikolajczak, K. L., Bagby, M. O., and Wolff, I. A. 1965. Alkaline isomerization of methyl crepenynate. J. Am. Oil Chemists' Soc. 42(3), pp. 243-245.

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VanEtten, C. H., Nielsen, H. C., and Peters, J. E. 1965. A crystalline polypeptide from the seed of Crambe abyssinica. Phytochemistry 4(3), pp. 467-473.

Industrial Utilization of New Oilseeds

Barclay, A. S.,[1] and Earle, F. R. ([1]USDA Crops Res. Div., Beltsville, Maryland). 1965. The search for new industrial crops. V. The South African Calenduleae (Compositae) as a source of new oilseeds. Econ. Botany 19(1), pp. 33-43.

Gentry, H. S.,[1] and Miller, R. W. ([1]USDA Crops Res. Div., Beltsville, Maryland). 1965. The search for new industrial crops. IV. Prospectus of Limnanthes. Econ. Botany 19(1), pp. 25-32.

Kinman, M. L.,[1] and Earle, F. R. ([1]USDA Crops Res. Div., College Station, Texas). 1964. Agronomic performance and chemical composition of the seed of sunflower hybrids and introduced varieties. Crop Sci. 4(4), pp. 417-420.

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Wolff, I. A., and Miwa, T. K. 1965. Effect of unusual acids on selected seed oil analyses. J. Am. Oil Chemists' Soc. 42(3), pp. 208-215.

Industrial Utilization of New Fiber Plants

Clark, T. F., and Wolff, I. A. 1965. A search for new fiber crops. VIII. Sulfate pulping of kenaf (*Hibiscus cannabinus*). Tappi 48(6), pp. 381-384.

AREA NO. 10: SUGARCANE
PROCESSING AND PRODUCTS - NORTHERN REGION

Problem. Quotas established by the Sugar Act effectively prevent the accumulation of surpluses by limiting production to estimated requirements at stable, low prices for sugar. Prices received by farmers of the United States and Puerto Rico for sugarcane are based upon the recoverable sugar content of the cane; and the rising costs of production and processing make imperative the more efficient recovery of increased amounts of sugar to provide adequate returns for both processors and growers. Currently, recovery of 75 percent of the total sugar in the cane is considered satisfactory in Louisiana, and about 83 percent in Puerto Rico and Hawaii. Improved processing methods could increase the recoverable sugar to at least 85 percent in Louisiana and over 90 percent in other areas. The development of more efficient processing methods depends in turn upon the acquisition of adequate data on the quantitative composition of juices extracted from sugarcane, and of materials processed to recover sugar.. The chemical industry provides a promising potential for the utilization of additional sugar since more than 15 billion pounds of chemical products are produced annually and sold to every section of American industry. More information is needed on the chemistry and properties of products from sugar to expand their utilization and on the application of these derivatives in the production of plastics, protective coatings, emulsifiers, detergents, and the like.

USDA AND COOPERATIVE PROGRAMS

The major part of the Department's research program on sugarcane processing and products is maintained at the Southern Utilization Research and Development Division, New Orleans, Louisiana. At the Northern Division, Peoria, Illinois, the Department has maintained a long-term continuing program involving microbiologists and biochemists engaged in basic and applied research on the fermentative conversion of sugar to industrially useful organic acids. Since the objectives have been achieved, this program was terminated with completion of the current project. No further studies are planned.

The Federal program at Peoria, Illinois, totals .6 professional man-year, all of which is devoted to new and improved products, specifically, fermentative conversion of sugar to α-ketoglutaric and 2-ketogluconic acids. This research has been completed.

In addition, the Department, through the Northern Division, sponsors research in this area under a grant of PL 480 funds to the Institute of Biological Chemistry, University of Rome, Rome, Italy, for studies on the preparation and characterization of dextran derivatives (5 years, 1961-1966). This research is under the subheading, new and improved products.

PROGRAM OF STATE EXPERIMENT STATIONS

Station sugarcane utilization research begins with studies directed to obtaining new, early maturing or cold-tolerant varieties which have a high yield and are adaptable to standard milling procedures and extends to work directed to preparation of flavored sirups for food use.

Most of the basic and exploratory research is carried out at the Puerto Rico station. Use of ion-exchange procedures for the production of sugars that may be utilized without further purification is under continuing study. Other work in progress involves: development of pilot-plant fermentation procedures for fermenting molasses mashes to produce rum; development of distillation procedures for high efficiency rum distillation; search for new strains of yeast for use in fermentation of blackstrap molasses and other materials derived from sugarcane; and determination of factors affecting the sucrose content of cane.

The study which was carried out in cooperation with USDA and which pertained to utilization of sorgo juice for sirup and sugar production is being terminated.

Indiana station research seeks to synthesize analogues of important metabolic sugars wherein hetero atoms such as sulfur, selenium or nitrogen replace the normal oxygen atom. Sugar analogues and their derivatives will be tested for usefulness as medicines or as agricultural chemicals.

The research effort on utilization of sugarcane is 4.9 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. New and Improved Products

1. Fermentative production of α-ketoglutaric and 2-ketogluconic acids. Experiments indicated that the Krebs cycle was responsible for formation of α-ketoglutaric acid by Pseudomonas sp. Therefore, experimental yields obtained under the best conditions are, in fact, close to the theoretical maximum of 54 percent. Further work on production of this acid is not desirable, since this yield would be too low to interest industry.

On the other hand, the process for 2-ketogluconic acid, developed collaterally in the course of this project, continues to appear very promising for industrial adoption. Crystalline calcium 2-ketogluconate was isolated from runs in 20-liter fermentors in a yield of 63 percent and in near 100-percent purity.

All studies specified in this project have now been completed. No further research on fermentative conversion of sugar to industrially useful products is planned.

2. Studies on dextran derivatives. Progress has been made on study of the kinetics and mechanism of reaction of (a) the controlled depolymerization of dextran by Fenton's reagent and (b) the interaction of dextran with gamma-globulin. Kinetics of reaction have been established for systems involving dextran derivatives with the model protein lysozyme, with certain dyes, and with metal salts that form complexes. Ultracentrifugation and electrophoretic examination indicated that phosphomannan Y-2448 has very narrow size distribution and therefore is a most exceptional polysaccharide. This research is being conducted under a PL 480 grant by the Institute of Biological Chemistry, University of Rome, Rome, Italy. In view of the termination of research on cane sugar at the Northern Division, further progress on this PL 480 project will be reported in Area 5, Wheat and Corn, Fermentative Conversion to New Industrial Feed and Food Products.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

New and Improved Products

Misenheimer, T. J., Anderson, R. F., Lagoda, A. A., and Tyler, D. D. 1965. Production of 2-ketogluconic acid by Serratia marcescens. Appl. Microbiol. 13(3), pp. 393-396.

Mondovi, B., Wolf, A. M., Turini, P., and Rossi-Fanelli, A. (Institute of Biological Chemistry, University of Rome, Rome, Italy). 1964. Studies on dextran and dextran derivatives. Note V. Stability to heat of dextran in aqueous solution. Ital. J. Biochem. 13(6), pp. 401-405.*

*Research supported by PL 480 funds.

AREA NO. 11: FORAGES AND FEED PROCESSING AND PRODUCTS - NORTHERN REGION

Problem. The demand for livestock in the United States will increase 45 percent by 1975. There is an increasing demand for processed forages in European and Asiatic markets. Fresh forage crops are the richest natural source of many nutrients for farm animals. The bulk of forages, however, is preserved so inefficiently by haymaking and ensiling that 10 to 50 percent of the dry weight, and much larger fractions of the most valuable nutrients, are lost before the animals eat them. Dehydration is now the only practical means of preserving the high nutritional value. Poultry and swine producers are aware of the value of dehydrated forage, but restrict their consumption because of high fiber and growth-inhibitor content. The livestock breeder needs forage products tailored to specific animals, and the forage producer must adapt to his needs to sell.

Basic and applied utilization research are necessary to produce: (1) nutritional juice and low-fiber, high-protein feed for non-ruminant animals; (2) fiber products which have been cheaply treated to make them easily digestible for ruminants; (3) growth-stimulating supplements for ruminants based on the biologically active fiber-digestion factor, and growth-promoting factor in forage. Further, new products should be adaptable to mechanical feeding. Improved handling will encourage farmers to put high-value land now producing surplus crops into forages.

USDA AND COOPERATIVE PROGRAMS

The major part of the Department's research program on forages is maintained at the Western Utilization Research and Development Division, Albany, California. At the Northern Division, Peoria, Illinois, the Department has a short-term program involving one organic chemist engaged in research to isolate and identify the toxic component(s) of tall fescue grass responsible for a cattle disease known as "fescue foot." This research is cooperative with the Kentucky State Experiment Station which furnishes toxic and nontoxic fescue grass for chemical study and conducts bioassays of fractions and components isolated from fescue at the Northern Division. Liaison is maintained with the fescue breeding program of the Field Crops Research Branch, ARS, through the Agronomy Department of the University of Kentucky and with the Department's Pharmacology Laboratory at the Western Division.

The Federal program at Peoria, Illinois, totals 1.3 professional man-years, all of which is devoted to chemical composition and physical properties.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations conduct an extensive program of both basic and applied research on forage utilization. Much of the research is interdisciplinary and often involves several departments.

One major segment of the research effort is devoted to determining the chemical composition of forages and evaluation of the relationship between chemical composition of certain forages and their nutritive value for farm animals. Evaluation of the effects of certain agronomic, cultural, processing and handling practices on composition, palatability and nutritive value of forages receives much research attention. Fiber content and utilization of fiber by swine, cattle, sheep and poultry affect the value and use of forages. Methods of isolating and analyzing for fiber are being developed. Investigation of normal and abnormal rumen fermentations of forages is fundamental to maximum utilization.

Careful studies of specific constituents of forages are being undertaken. Determination of certain minor elements found in forages is important both from nutrition and toxicity standpoints. Protein content and quality merit special attention along with determination of amino acid values and unknown growth factors. Leaf organic acids and proteins are investigated in detail in an effort to increase our understanding of their biosynthesis and properties in relation to growth of forage plants.

Due to the economic importance of forages in animal feeds, development of means for evaluation of the nutritive quality of forages has become an important field of study. New and more accurate or rapid chemical procedures are being sought.

Development of forage handling and processing systems to minimize labor costs has led to increased research on forage processing methods. Fermentation characteristics of and animal response to forages which have been wilted, chopped, pelleted, ensiled or dehydrated are being determined. Small-scale ensiling systems are being used to evaluate various silage preservatives. Methods of dehydrating alfalfa are being studied and the economic feasibility of dehydration is being investigated.

The total research effort devoted to forage utilization is about 22.7 professional man-years.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Fescue toxicity. Work on this project is now completely reoriented to test the hypothesis that mycotoxins may be the cause of fescue foot in cattle. The Northern and Western Divisions and Kentucky AES are cooperating

in this study and maintaining close contact for planning and exchange of information.

Survey of toxic fescue hays for types of fungi present has been initiated. Some 35 strains of fungi from fescue hays have been identified. Fescue hay inoculated with selected mold organisms and then incubated, and extracts of several fungi and of toxic hay have been sent to the Western Division for use in developing and evaluating small animal bioassay methods.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

Yates, S. G., and Tookey, H. L. 1965. Festucine, an alkaloid from tall fescue (Festuca arundinacea Schreb.): Chemistry of the functional groups. Australian J. Chem. 18(1), pp. 53-60.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1	Corn, wheat, and other cereal crop utilization investigations--Northern region.			
N1 1-58 (Rev. 2)	Operation and improvement of a culture collection of molds, yeasts, bacteria, and actinomycetes to provide a reservoir of authentic microorganisms for use in making antibiotics, vitamins, chemicals, polymers, assays, and identifications of importance to the national welfare.	Peoria, Ill.	Yes	5-A-1
N1 1-172(C)	Investigation of the chemical reactions of periodate-oxidized starch (dialdehyde starch) in solutions involved in its potential practical applications as a basis for improving and enlarging its industrial utility.*	St. Paul, Minn.	No	
N1 1-175	Investigations on the carotenoid pigments of wet- and dry-milled fractions from corn, including high-amylose types, and of yellow-endosperm sorghum to provide data basic to the most effective utilization of industrial products and fractions from corn and sorghum in feeds.	Peoria, Ill.	Yes	3-A-2
N1 1-176(C)	Polymerization investigations on selected fermentation acids from cereal grains, and on selected fatty acid derivatives from linseed, soybean, and mustard oils, for conversion of these agricultural products to plastics and resins.*	Tucson, Ariz.	No	
N1 1-178	Investigations on the molecular size and state of aggregation of the amylose and amylopectin components of high-amylose corn starches to provide information basic to industrial utilization.	Peoria, Ill.	Yes	4-A-2
N1 1-179	Basic studies on the chemical structure of the amylose and amylopectin components of high-amylose corn starches to provide information needed for effective industrial utilization of these new starches.	Peoria, Ill.	No	
N1 1-180(C)	Investigation of factors required by *Bacillus popilliae* and *Bacillus lentimorbus* to produce large and vigorous populations of cells in grain-based media for the mass production of spore dusts to control Japanese beetle infestations.*	East Lansing, Mich.	Yes	5-C-1
N1 1-181	Studies of the effects of conditioning treatments of wheat on morphological and histochemical characteristics of milled fractions to provide information basic to the production of industrially useful fractions from wheat.	Peoria, Ill.	Yes	2-C-2
N1 1-182	A comprehensive study of sexual agglutination in yeasts as a basis for developing new yeasts and new processes for the fermentative conversion of cereal grains to new products.	Peoria, Ill.	Yes	5-A-2
N1 1-183	Exploratory studies to convert wheat flour into water-resistant, plasticlike chemical derivatives having properties suitable for industrial use in structural and insulating products and in molding compositions.*	Peoria, Ill.	Yes	1-B-1
N1 1-184	Chemical conversion of wheat flour into a variety of hydrophilic polymers having a wide range of solubilities and viscosities in aqueous dispersions to meet specific industrial requirements for sizes, adhesives, and thickeners.	Peoria, Ill.	Yes	2-B-3

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-186(C)	Studies on the reaction of acetylene with starch and starch-derived products as a basis for development of new products from cereal grains.*	Tucson, Ariz.	Yes	1-A-2
N1 1-187	Isolation and characterization of physiologically active nonprotein nitrogenous substances in corn and corn-milling products as a basis for applied processing studies to increase the use of corn.	Peoria, Ill.	Yes	3-A-1
N1 1-190(C)	Investigations on methods for the chemical preparation and characterization of amino derivatives of cereal starches by replacement of nonglycosidic hydroxyl groups to obtain new starch products having increased stability to water, dilute acids, and alkali.	Columbus, Ohio	Yes	1-B-6
N1 1-191	Basic taxonomic studies on straight and flexuous streptomycetes of importance to the production of plant antibiotics by fermentation of cereal grains.*	Peoria, Ill.	Yes	5-A-2
N1 1-192	Screening microorganisms that may be grown on cereal-based media to produce antibiotics effective against selected fungal diseases in plants, thus providing new fermentation outlets for cereal grains.*	Peoria, Ill.	Yes	5-C-2
N1 1-194	Search for microorganisms and a fermentative process to convert cereal grain products to xanthophylls that induce desirable pigmentation of poultry products when added to feed.	Peoria, Ill.	Yes	5-D-1
N1 1-195(C)	Investigations on the alkaline desulfurization of wheat gluten proteins to provide a basis for developing improved modifications of wheat products having utilization potential.	Lafayette, Ind.	Yes	2-A-2
N1 1-196	Chemical investigations on amylomaize selections to guide corn breeders in the development of commercial hybrids containing high-amylose starch for industrial use.	Peoria, Ill.	Yes	4-A-1
N1 1-197	Engineering process studies on the acid modification of wheat flour to prepare water-dispersible polymeric products and to make quantities available for product evaluation for use as sizes, adhesives, and thickeners.*	Peoria, Ill.	Yes	2-B-1
N1 1-199(C)	Investigations on control of the chemical hydrolysis of cereal proteins to provide a basis for development of processes to yield polypeptides suitable for industrial uses.*	Chicago, Ill.	Yes	2-A-2
N1 1-200	Evaluation of modified cereal flours and starches as sizing agents, coating adhesives, and wet-end additives for paper in large-scale, high-speed continuous runs.	Peoria, Ill.	Yes	2-B-5
N1 1-202	Isolation and characterization of the toxic principle in tall fescue responsible for a cattle disease known as "fescue foot" to provide basic information for increased use of this forage.*	Peoria, Ill.	Yes	11-A-1
N1 1-203(C)	Investigations on the control of a selected complex reaction of starch or related carbohydrates through controlled fluid flow dynamics and reaction conditions to provide a basis for process design and improvement leading to increased utilization of cereal grains.	Baltimore, Md.	Yes	1-A-7

* Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-204	Chemical investigations on the molecular structure of the protein, glutenin, present in wheat gluten as a basis for increased industrial utilization of this raw material.	Peoria, Ill.	Yes	2-A-1
N1 1-205(C)	Investigations on reactions of difunctional mercaptans with dextrose, starch, or related carbohydrates to form polymers having potential industrial value.	Tucson, Ariz.	Yes	1-A-2
N1 1-206(C)	Evaluation of beta-carotene product from fermentation of grain-based media with Blakeslea trispora as vitamin A source for poultry and swine.*	East Lansing, Mich.	No	
N1 1-207	Investigations on the enzymatic modification of wheat flour and flour fractions by combined amylases and proteases to provide pastes of suitable viscosities for use as surface sizes and coating agents for paper.*	Peoria, Ill.	Yes	5-B-2
N1 1-208	Investigations on the conversion of cereal grains to economical and efficient soluble fermentation substrates through the action of microbial enzymes, as a basis for increasing the use of these grains by the fermentation industry.	Peoria, Ill.	Yes	5-B-1
N1 1-209	Investigations on the applicability and evaluation of chemically modified cereal grain flours and fractions as ingredients, agents, and adhesives in pulp and paperboard products as a basis for increasing industrial use of cereal grains.	Peoria, Ill.	Yes	1-B-2,7; 2-B-5
N1 1-210	Investigations on the preparation of water-dispersible hetero-derivatives of starch to obtain products having a wide range of properties for the production of adhesives, sizings, and other additives for applications in paper and related industries.	Peoria, Ill.	Yes	1-B-6
N1 1-211	Chemical reaction studies on wheat gluten and its component proteins seeking methods of modification to give properties better suited for industrial uses.	Peoria, Ill.	Yes	2-A-2
N1 1-212	Investigations on the production of low-density plastic foams from starch-derived glucosides and related starch derivatives as a basis for increasing the industrial utilization of cereal starches.	Peoria, Ill.	Yes	1-B-3
N1 1-213	Pilot-plant investigations on wheat dry-milling and fractionation methods for producing a wide variety of products for use in foods, feeds, and industrial products.	Peoria, Ill.	Yes	2-C-1
N1 1-214(C)	Engineering studies on the application of pneumatic fluidization to the reactions of wheat flour with hydrogen chloride as a basis for producing sizing agents for paper.	Ames, Iowa	Yes	2-B-2
N1 1-215	Investigations of the reaction of dialdehyde starch with casein, soybean protein, soy flour and dried animal blood for the production of improved wood adhesives.	Peoria, Ill.	Yes	1-B-5
N1 1-216	Pilot-plant investigations of dry-milling operations to obtain increased yields of prime goods and oil from old and artificially dried corn and to develop a prototype corn degerminator having improved corn degerminating characteristics for production of higher quality dry-milled products.	Peoria, Ill.	Yes	3-B-1

* Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-217	Investigation of methods for producing microbial polysaccharides from cereal grains by continuous fermentation to reduce production costs allowing increased utilization of these potentially useful gums.	Peoria, Ill.	Yes	5-B-3
N1 1-218(C)	Stabilization of vegetative cells of Bacillus popilliae grown on cereal-based media for use as an infecting agent against the Japanese beetle.	Manhattan, Kans.	Yes	5-C-1
N1 1-219(C)	Study of role of enzymes and enzyme activity in the formation of spores of Bacillus popilliae and Bacillus lentimorbus as a basis for the mass production of biological insecticides by fermenting cereal grain.	East Lansing, Mich.	Yes	5-C-1
N1 1-220(C)	The transfer of genetic determinants of sporulation from one microorganism to another, as a basis for applied studies on the fermentative production of spore dusts for the control of Japanese beetle infestations.	Minneapolis, Minn.	Yes	5-C-1
N1 1-221(C)	Study of the sporulation factor produced by bacilli and its possible use in Bacillus popilliae and Bacillus lentimorbus to develop a fermentation process for the production of spore dust to control Japanese beetle infestations.	Urbana, Ill.	Yes	5-C-1
N1 1-222(C)	Studies on the mechanism and kinetics of radiation and ceric ion induced grafting of cereal starches with vinyl-type monomers previously shown in exploratory studies to graft readily and efficiently with promise for new industrial outlets for starch.	Menlo Park, Calif.	Yes	1-B-4
N1 1-223(C)	Development studies on the semi-pilot-plant scale production of cereal grain xanthides and their use and evaluation in making corrugating board and linerboard for corrugated boxes.	Columbus, Ohio	Yes	1-B-2
N1 1-224	Development of methods and processes to reduce viable microorganisms in wheat flour as it is produced in the mill.	Peoria, Ill.	Yes	2-C-3
N1 1-225	Investigations on the development of new fermented wheat foods through the use of Oriental-type food molds as a basis for increasing export markets for U. S. wheats.	Peoria, Ill.	Yes	5-D-2
N1 1-226	Investigations on formation and properties of amino and peptide derivatives of starch to provide a basis for the development of industrially useful products from cereal grains.	Peoria, Ill.	Yes	1-A-3
N1 1-227	Investigations on the conversion of cereal xanthates to xanthides in physical forms suitable for use in papermaking.	Peoria, Ill.	Yes	1-B-1
N1 1-228(C)	Investigations on the interaction of "V" amylose with small molecules to provide basic information on the helical structure of amylose from high-amylose corn starch.	Tempe, Ariz.	Yes	4-A-2
N1 1-229	Investigations on processing methods for wheat to minimize radioactive contamination in milling products.	Peoria, Ill.	Yes	2-C-4
N1 1-230(C)	Investigations on the synthesis of terminal C4-modified maltooligosaccharides for use in studying enzyme modifications of cereal starches.	Carbondale, Ill.	Yes	1-A-1

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-231(C)	Stabilization of beta-carotene in dried mycelium and in extracted form as a contribution to commercialization of beta-carotene produced by fermentation of cereal grain.	Cambridge, Mass.	Yes	5-D-1
N1 1-232(C)	Investigations on the vinylation of methyl glucoside by reaction with acetylene and on the properties and reactions of the products as a basis for development of new industrial outlets for cereal grains.	Tucson, Ariz.	Yes	1-A-2
N1 1-233(Gr)	Studies on the types and variations of starch granules within the endosperm of genetically different high-amylose corns to provide fundamental information important to the processing and utilization of high-amylose corn.	Lincoln, Nebr.	Yes	4-A-2
N1 1-234(Gr)	Investigations of two-phase submerged fermentation processes as means for increasing yields and/or concentrations of products obtained by fermentation of cereal grains.	Ithaca, N. Y.	Yes	5-B-3
N1 1-235(C)	Investigation of the morphological changes involved in the transition of Bacillus popilliae from vegetative cells to spores for controlling Japanese beetle infestations.	Houston, Tex.	Yes	5-C-1
N1 1-236(C)	Investigation on the isolation and characterization of phenolic pigments of grain sorghum to provide basic information related to the discoloration of milled sorghum and its starch.	Bloomington, Ind.	Yes	3-A-2
N1 1-237	Investigation of the characteristics and classification of microorganisms of the section Dubiorugorhizopus of the genus Rhizopus of the family Mucoraceae, as tools for use in the development of fermentations utilizing cereal crops.	Peoria, Ill.	Yes	5-A-2
N1 1-238(C)	Studies on kernel properties and milling and fractionation characteristics of wheats exhibiting a range of kernel hardness and protein content to provide information basic to the production of a range of products for industrial uses and application in baking.	Lincoln, Nebr.	Yes	2-C-1
N1 1-239(Gr)	Basic investigations on the chemical and molecular structure of amyloglucosidases with emphasis on relationship to enzyme formation and action to provide information applicable to the production and use of these enzymes in the utilization of cereal grains.	Lincoln, Nebr.	No	
N1 1-240	New microbial polysaccharides of commercial value produced from cereal grains: Characterization and structural analysis of previously selected polysaccharides and screening for additional polysaccharides with new and broader range of applicability.	Peoria, Ill.	Yes	5-B-4
N1 1-241	Investigations on molecular structure, aggregation, and interactions of wheat gluten proteins and their chemical modifications to provide basic information related to industrial utilization of wheat.	Peoria, Ill.	Yes	2-A-1
N1 1-242	Chemical transformations of maltose and dextrose to determine differences in reactivity and to produce new compounds of possible industrial use from these cereal starch-derived sugars.	Peoria, Ill.	Yes	1-A-1
N1 1-243	Modification of fermentations by transfer of genetic material in microorganisms.	Peoria, Ill.	Yes	5-A-3

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N1 1-244	Exploratory studies on the chemical and physical modification of high-amylose corn starches to improve their applications as coatings and sizings for industrial use.**	Peoria, Ill.	Yes	4-B-1
N1 1-245(C)	Development of optimal papermaking processes using cereal grain xanthides made from starch, ground whole grain, flour, bran, shorts, and other dry-milled grain products in blends with wood pulp to produce linerboard, corrugating media, and bag papers.	Columbus, Ohio	Yes	2-B-4
N1 1-247	Exploratory studies on the chemical synthesis and characterization of crosslinked starch derivatives having potential value as paper additives for improvement of tear, stretch, and moisture stability of paper products and for upgrading properties of boxwood, insulating board, and other structural materials.	Peoria, Ill.	Yes	1-B-6
N1 1-248(C)	Preparation and evaluation of selected starch graft copolymers for industrial use in plastic products and industrial coatings.	Menlo Park, Calif.	Yes	1-B-4
N1 1-249(C)	Investigations on the preparation of plastic foam from selected starch polyol derivatives and their evaluation in industrial applications.	Minneapolis, Minn.	Yes	1-B-3
N1 1-250(Gr)	The reaction of vinyl ethers with carbohydrates, especially D-glucose and starch.	Columbus, Ohio	Yes	1-A-4
N1 1-251(Gr)	Basic studies on the relation of viscoelastic properties of amylose sheets and films to structure and function of added plasticizers.	Princeton, N. J.	Yes	4-A-3
N1 1-252(Gr)	Basic investigations on the organic chemistry of unsaturated and sulfur-containing carbohydrates to provide a basis for the development of new reactions and derivatives of cereal grain starches and related sugars.	Columbus, Ohio	Yes	1-A-4
N1 1-253	Studies on the production of mycotoxins by Aspergillus flavus and related molds to provide basic information for processing grain into feeds.	Peoria, Ill.	Yes	5-A-4
N1 1-254(C)	Development of improved methods for preserving microorganisms that cannot be satisfactorily lyophilized for use in the fermentative conversion of cereal grain into industrial products.	Rockville, Md.	Yes	5-A-1
N1 1-255	Investigation of the sporulation of milky disease bacteria in vivo and in vitro as a basis for the development of a fermentation process for the production of a pesticidal agent against the Japanese beetle.	Peoria, Ill.	Yes	5-C-1
N1 1-256	Exploratory studies on the preparation of new and novel products from unmodified cereal starches and thin-boiling starches by graft copolymerization with selected vinyl and acrylic monomers.	Peoria, Ill.	Yes	1-A-5
N1 1-257	Basic studies on the relation of film properties of amylose to molecular organization and structure in order to provide information needed in improving the preparation and properties of films and coatings from amylose and high-amylose starch.**	Peoria, Ill.	Yes	4-A-3
N1 1-258(C)	Application of antimetabolites for selecting mutants of Blakeslea trispora to enhance yields of beta-carotene by fermentation of cereal grain and other agricultural products.**	Chicago, Ill.	Yes	5-D-1

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-259	Exploratory investigations on the conversion of cereal grain carbohydrates to industrial materials through the use of microbial enzyme systems.**	Peoria, Ill.	No	
N1 1-260(C)	Preparation, characterization, and chemical modification of polypeptides derived from cereal proteins to yield products of potential industrial interest as a basis for increasing the utilization of cereal grains.**	Chicago, Ill.	Yes	2-A-2
N1 1-261(C)	Establishment of practical conditions for the application of acid-modified flour as a surface-sizing agent for paper on a semicommercial paper machine.**	Berlin, N. H.	Yes	2-B-1
N1 1-262(C)	Evaluation of cyanoethylated modified corn starches for application in paper processes as a basis for the development of expanded markets for cereal grain products.**	Kalamazoo, Mich.	Yes	1-B-6
N1 1-263	Study of plant-protective and chemical properties of antibiotics produced by fermentation of cereal-based media with streptomycetes and correlation of taxonomic characteristics of antibiotic-producing microorganisms with antibiotic identities.**	Peoria, Ill.	Yes	5-C-2
N1 1-264	Engineering studies on modifications of cereal flours and starches to prepare useful polymeric products and to evaluate the products and processes.**	Peoria, Ill.	Yes	1-B-2
N1 1-265(Gr)	Basic investigations on the mechanical and viscoelastic properties of corn kernels as influenced by tempering conditions employed in the dry-milling process.**	University Park, Pa.	Yes	3-B-1
N1 1-266	Investigations on the application of NMR spectroscopy to grain constituents and derived products to provide information on chemical and molecular structure pertinent to utilization research.**	Peoria, Ill.	Yes	1-A-6
N1 1-267(C)	Investigations on the protein content, amino acid composition, and biological feeding value of hybrid grain sorghums and selected milling products as a foundation for improvements in the utilization of grain sorghum in foods and feeds.**	Manhattan, Kans.	No	
N1 1-268(Gr)	Synthesis and degradation of O-glycosides of hydroxy amino acids that form protein to carbohydrate linkages in glycoproteins to provide basic information on the reactions of carbohydrates in proteinaceous compounds and specific information useful for characterizing cereal glycoproteins.**	Milwaukee, Wisc.	Yes	2-A-2
N1 1-269(Gr)	Investigations on the principles of disc electrophoresis as a method for large-scale separation of proteins and enzymes.**	Manhattan, Kans.	Yes	2-A-1
N1 1-270(Gr)	Basic studies on heat, mass, and momentum transport in cereal starches and flours to provide information for the design of more economical processes for chemical and physical modifications.**	Ames, Iowa	No	
N1 1-271(Gr)	Investigations on amylases from bacteria and on their action patterns and products found on amylolysis of starch and related substrates.**	Ames, Iowa	No	
N1 1-272(Gr)	Basic research on the preparation and characterization of sugars containing carbon bound nitrogen, phosphorus, and sulfur.**	Lafayette, Ind.	Yes	1-A-4

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-273(Gr)	Basic studies on the mechanism and effects of chemical cleavage of disulfide bonds in wheat and corn endosperm proteins to provide information for improving the processing and utilization of cereal grains.**	Lafayette, Ind.	Yes	2-A-2
N1 1-274(C)	Exploratory studies on the suitability of starch derivatives as protective colloids and binders for use in water-emulsion paints.**	Columbus, Ohio	No	
N1 1-276	Exploratory investigations on the enzymatic conversion of cereal starches and glucose to unique glucosides, polyols and isomerization products of glucose as a basis for increasing the industrial utilization of cereal grains.**	Peoria, Ill.	Yes	5-B-1
N1 1-277	Study of principles associated with tall fescue which cause toxicity in cattle or limit utilization of the nutrients of this grass.**	Peoria, Ill.	Yes	11-A-1
N4 2	Soybean and other oilseed utilization investigations --Northern region.			
N4 2-74	Basic investigations on the selective hydrogenation of linolenic acid as a means of improving the flavor stability of edible soybean oil.*	Peoria, Ill.	Yes	7-B-1
N4 2-77 (Rev.)	Engineering investigations on the production of cyclic fatty acids from linseed oil.	Peoria, Ill.	Yes	6-B-1
N4 2-78	Chemical and physical characterization studies on the electrophoretically and chromatographically separated proteins found in defatted soybean meal whey and on their associated nonprotein components to provide information basic to improving the industrial and feed value of whey proteins.	Peoria, Ill.	Yes	8-A-1
N4 2-83(C)	Studies on the effect of linseed oil coatings on the durability of air-entrained concrete and its resistance to freeze-thaw cycles for evaluating the use of this oil in protecting this type of concrete against deterioration.	Manhattan, Kans.	Yes	6-C-2
N4 2-84 (Rev.)	Exploratory investigations on products obtained by reacting linseed and soybean oils and their fatty acids with selected polyols and other hydrophilic reagents, and characterization of the products for utility as water-soluble paint vehicles.	Peoria, Ill.	Yes	6-C-3
N4 2-85	Investigations on the preparation, properties, and reactions of aldehyde oils obtained by the ozonolysis of soybean, linseed and erucic acid oils, as a basis for their increased industrial utilization.	Peoria, Ill.	Yes	7-C-1,2
N4 2-86	Investigations on new polymeric products from aldehydic materials obtained by the ozonization of soybean and linseed oils, as a basis for increased industrial utilization of these oils.	Peoria, Ill.	Yes	7-C-2
N4 2-87	Engineering studies on the production of aldehyde oils from soybean, linseed, and other unsaturated vegetable oils.	Peoria, Ill.	Yes	7-C-1
N4 2-88	Basic investigations on the chemical reactions of soybean and linseed oils and their fatty acids with ethylene and other commercially available olefinic compounds to produce new products having potential industrial value.	Peoria, Ill.	Yes	6-B-1

* Discontinued during reporting year.
** Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N4 2-89	Studies on edible soybean oil: Improvements achieved by mixing soybean oil with other edible oils.	Peoria, Ill.	Yes	7-A-2,B-2
N4 2-90(C)	Investigations on flatus caused by ingestion of soybean foods as related to the development of foreign-type foods to expand export markets.	Urbana, Ill.	Yes	8-B-1
N4 2-91	Preparation of new derivatives from soybean and linseed fatty acids or oils containing vinyl groups capable of polymerizing to form new polymers and copolymers of potential industrial value in coatings and related fields.	Peoria, Ill.	Yes	6-C-3
N4 2-92(C)	Preparation and evaluation of heterogeneous catalysts for specificity in hydrogenation of linolenate in soybean and linseed oils to increase industrial and food applications.	Chicago, Ill.	Yes	7-B-1
N4 2-93(C)	Basic investigations on heterogeneous catalysts for the selective hydrogenation of linolenate in soybean oil to provide basic information for increased food applications.	New Brunswick, N. J.	Yes	7-B-1
N4 2-94(C)	Investigations on the conversion of aldehyde oils to coating compositions for wood and metal as a basis for developing new industrial products from linseed and soybean oils.	Fargo, N. Dak.	Yes	6-C-3
N4 2-95	Investigations on improving flavor stability of edible soybean oil by concurrent ester interchange of the oil and selective extraction of trilinolenin.	Peoria, Ill.	Yes	7-B-1
N4 2-96	Exploratory investigations on the flavor, texture, and color of soybeans, soybean fractions and products used in foods and protein supplementation to increase the use of soybeans and soybean products in foreign markets.	Peoria, Ill.	Yes	8-B-1
N4 2-97(C)	Basic studies on the organo complexes of transition metals as homogeneous catalysts for hydrogenation of soybean oil.	Urbana, Ill.	Yes	7-B-1
N4 2-98	Investigations on heat gelation of alcohol-washed soybean protein as a basis for developing new food and industrial uses for this protein.	Peoria, Ill.	Yes	8-A-1
N4 2-99(C)	Studies on the effect of linseed oil coatings on the curing and durability of concrete, and evaluation of selected linseed oil compositions for this potentially new use.	Manhattan, Kans.	Yes	6-C-2
N4 2-100(C)	Investigations on the preparation of copolymers of linseed oil and vinyl monomers suitable for emulsion paints.	Menlo Park, Calif.	Yes	6-C-3
N4 2-101(C)	Investigations on the chemical and physical properties of poly(ester-acetals) and poly(amide-acetals) derived from soybean and linseed oils and of the bonds formed between them and various substrates after crosslinking.	Dedham, Mass.	Yes	7-C-2
N4 2-102	Microbial modification of fatty acids to produce derivative long-chain acids of potential industrial utility.	Peoria, Ill.	Yes	5-A-3
N4 2-103	Basic studies on the water permeability and water sensitivity of linseed oil films, as a basis for increasing the utilization of linseed oil in paints.**	Peoria, Ill.	No	

** Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N4 2-104	Investigations on the effect of emulsion and oil viscosities, and of particle size in oil-in-water emulsions on the film-forming properties of linseed oil, as a basis for increasing the utilization of linseed oil in paints.**	Peoria, Ill.	Yes	6-C-1
N4 2-105(C)	Pilot preparation of materials for developmental investigations on aldehyde oils and related products derived from soybean and linseed oils.**	Minneapolis, Minn.	Yes	7-C-1
N4 2-106	Investigation of chemical and molecular structure of vegetable oils and their derivatives from soybeans, flax and other commodities of the Northern Region by mass spectrometry to obtain information pertinent to utilization research.**	Peoria, Ill.	Yes	7-A-1
N4 2-107	Investigation of selective heterogeneous catalytic hydrogenation to provide information basic to increasing the edible use of soybean oil.**	Peoria, Ill.	Yes	7-B-1
N4 2-108	Basic investigations on homogeneous catalysts for selective hydrogenation of soybean and other linolenate oils for use in edible and industrial products.**	Peoria, Ill.	Yes	7-B-1
N4 2-109	Evaluation of edible soybean oil products by chemical, physical, and organoleptic methods to provide basic information for increasing applicability in food uses.**	Peoria, Ill.	No	
NU-0-0-1 (AID)	Engineering development of processes for converting soybeans to high-quality full-fat soybean flours for use in various developing countries of the world.**	Peoria, Ill.	Yes	8-B-2
N5 1	Sugars and sirups investigations.*			
N5 1-69	Investigations on the fermentative production of α-ketoglutaric acid from sugar or molasses to provide new industrial outlets for these agricultural materials.*	Peoria, Ill.	Yes	10-A-1
N5 5	New and replacement crops utilization investigations.			
N5 5-15 (Rev.)	Chemical screening to determine the amount and kind of fiber and accompanying constituents in selected plants, as a basis for discovering potential new domestic sources of fiber for pulp and papermaking.	Peoria, Ill.	Yes	9-A-5
N5 5-32 (Rev.)	Chemical survey of seed lipids from uncultivated domestic and foreign plants to discover sources containing economic amounts of industrially valuable constituents.	Peoria, Ill.	Yes	9-A-1
N5 5-33 (Rev.)	Characterization of selected fractions and chemical components of seeds of plant species containing favorable amounts of gross constituents to obtain more specific evaluation of their potential industrial importance than is afforded by screening analyses.	Peoria, Ill.	Yes	9-A-2,4
N5 5-41	Investigation of selected plants of the Hibiscus genus, with emphasis on kenaf and okra, to evaluate and develop fibrous products from annual plant sources having superiority or specific preferred properties for industrial use.	Peoria, Ill.	Yes	9-C-1

* Discontinued during reporting year.
** Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N5 5-44	Analytical investigations on proteins and other nitrogenous substances in meals from potential new oilseed crops, with emphasis on those bearing oils of high erucic acid content, to obtain fundamental information of value in their processing and utilization.	Peoria, Ill.	Yes	9-A-3
N5 5-47	Engineering studies on a process for converting Crambe abyssinica seed and closely related new oilseeds into oil and detoxified meal for evaluating the utilization potential of these new oilseed crops.	Peoria, Ill.	Yes	9-B-1
N5 5-48(C)	Investigations on the preparation of omega-amino tridecanoic acid from crambe oil, the production of polyamides from it, and evaluation of the polymers for industrial uses.	Birmingham, Ala.	Yes	9-B-2
N5 5-49	Investigation of the enzyme systems, bitter principles, pigments and other selected constituents of defatted crambe and closely related seed meals considered pertinent to seed processing and meal utilization as feed.**	Peoria, Ill.	Yes	9-A-3;B-1
N5 5-50	Investigations on the chemical modification of crambe oil, and of acids readily derived from it to prepare derivatives or chemical intermediates having properties desirable for industrial use.**	Peoria, Ill.	Yes	9-B-2
NU P-1	Pioneering Laboratory for Microbiological Chemistry.	Peoria, Ill.	Yes	5-A-3

** Initiated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1964 to June 30, 1965

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
(10)	Cereal and forage crops.			
UR-A7-(10)-7	Fundamental studies of enzyme systems isolated from Pseudomonas, to obtain information on the conversion of carbohydrates derived from cereal grains to organic acids having potential industrial value.***	Bangalore, India	No	
UR-A7-(10)-9	Collection and isolation of molds belonging to the order Mucorales, and classification of the isolates, in order to find microorganisms suitable for fermentative processes of importance in cereal grain utilization.	Allahabad, India	Yes	5-A-1
UR-A7-(10)-10	A study of survival and possible genetic change in industrially useful microorganisms subjected to lyophilization, to obtain basic information needed for the maintenance of culture collections for industrial fermentation of cereal grains.	Allahabad, India	Yes	5-A-1
UR-A7-(10)-20	Investigations on the preparation and characterization of new copolymers of cereal starch with other polysaccharides by heating mixtures in the dry state, to provide basic information for the development of new starch products suited for industrial applications.	Ahmedabad, India	Yes	1-B-6
UR-A7-(10)-25	Investigations on the separation of grain sorghum proteins into homogeneous protein components, to provide basic information for further characterization and application studies.	Bangalore, India	Yes	3-A-3
UR-A7-(10)-75 (Rev.)	Investigation of the distribution of aerobic actinomycetes in India, with particular emphasis on their isolation, characterization, antibiotic production, and preservation, for placement in the Culture Collection of the Agricultural Research Service as potential agents for the conversion of farm-produced raw materials to products useful to industry and the consuming public.**	Lucknow, India	No	
UR-A7-(10)-98	Investigations of methods for the chemical preparation and characterization of hydroxyethyl ethers of cereal starches prepared by partial replacement of specific hydroxyl groups to obtain new starch products with improved properties.**	Ahmedabad, India	Yes	1-B-6
UR-A10-(10)-27	Studies on the preparation and properties of graft copolymers of starch and dextrin obtained by reaction with vinyl monomers and epoxides, to provide a basis for increased industrial utilization of cereal grains.	Jerusalem, Israel	Yes	1-B-4
UR-A10-(10)-51	Fundamental studies on the mild oxidation of cereal grain starches by selected oxidizing agents for the determination of reaction mechanisms and the physical and chemical properties of modified starches of importance to their production and industrial use.	Jerusalem, Israel	Yes	1-A-10

** Activated during reporting year.
*** Cancelled.

PL 480 Research Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
UR-A11-(10)-17	Investigations to discover microorganisms that produce useful quantities of D-tartaric acid, and to explore laboratory processes for its production by fermentation of cereal grain products, as a basis for increasing the utilization of such products.**	Tokyo, Japan	Yes	5-B-5
UR-A11-(10)-19	Investigations on the development of a polarographic method for determining aldehyde and ketone carbonyl groups in products derived from cereal grain starches to facilitate the characterization of such polysaccharides as a basis for increasing their utilization.**	Sakai, Japan	No	
UR-A11-(10)-22	Investigations to discover microorganisms that produce useful quantities of mevalonic acid, and to develop an efficient process for the economic production of mevalonic acid by fermentation of cereal grain products, as a basis for increasing the utilization of such products.**	Tokyo, Japan	Yes	5-B-5
UR-E4-(10)-2	Search for lytic enzymes of microbial origin with activity on cell walls of bacteria, actinomycetes, molds, and yeasts to provide a basis for the development of new fermentation processes for the increased utilization of cereal grains.**	Liege, Belgium	Yes	5-A-3
UR-E8-(10)-6	Isolation of organic phosphorus derivatives found in the yeast Torulopsis utilis and elucidation of their structures, to provide new basic information on the fermentation of cereal products to industrial materials by yeasts.*	Helsinki, Finland	Yes	5-A-3
UR-E9-(10)-37 (Rev.)	Fundamental investigations of the proteolysis-inhibiting effects of cereal flours and starches, and of processing methods for minimizing such effects, to provide a basis for improved quality and increased utilization of cereal products.*	Paris, France	Yes	1-A-8
UR-E9-(10)-40	Investigations of the zein protein of corn: Fractionation and study of rheological and physical-chemical properties, chemical composition and structure, and problems of hydration and gelification of fundamental importance to the technology and industrial utilization of corn proteins.	Paris, France	Yes	3-A-3
UR-E9-(10)-42	A fundamental investigation of the physico-chemical alterations brought about in starches and their molecular constituents by gamma-radiations, to provide information needed for modification of starch properties and for the treatment of starch-containing products used industrially or in foods.	Paris, France	No	
UR-E9-(10)-56	Selection and mutation of strains of yeast capable of producing high quantities of sulfur-containing amino acids for use in increasing the efficiency of cereal-grain-based feeds deficient in these amino acids.	Paris, France	Yes	5-D-4

* Completed project.
** Activated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
UR-E15-(10)-21	Investigation of the growth factor (Vitamin B_{13}) of distillers' dried solubles through studies of methods of isolation and purification, mode of formation, and conditions of optimum production by yeast fermentation of cereal grains, to provide basic information for utilizing grains to produce this vitamin.	Milan, Italy	Yes	5-D-3
UR-E15-(10)-25	Investigations of the reaction of cereal starch dextrins with fatty acid chlorides and fatty amines, and evaluation of the products, to provide information important to increasing the utilization of wheat, corn, and sorghum.	Bologna, Italy	Yes	1-B-6
UR-E15-(10)-26	Investigation of the fermentative conversion of glucose to 5-ketogluconic acid through studies of a metabolic pathway in organisms of the Acetobacter genus, to obtain fundamental information for the utilization of grain products in the fermentative production of chemical intermediates.	Milan, Italy	Yes	5-A-3
UR-E15-(10)-32	Investigations on the conformation of glucopyranose rings in amylose corn starches and in linear and cyclic dextrins prepared from these starches, to provide basic information for the chemical modification of starch-derived products for the development of new uses.	Milan, Italy	Yes	1-A-9
UR-E19-(10)-18	Studies on the preparation of metal alkoxides of starch for use as intermediates in the synthesis of starch derivatives, to provide a basis for increasing the industrial utilization of cereal starches.**	Delft, The Netherlands	Yes	1-B-6
UR-E21-(10)-11	Investigations on the fermentative production of itatartaric acid from glucose, sucrose, or molasses to provide new industrial outlets for these agricultural materials.	Lodz, Poland	Yes	5-B-5
UR-E25-(10)-11	Isolation and characterization of yeasts for placement in the Culture Collection of the Agricultural Research Service, as potential agents for the conversion of farm-produced raw materials to products useful to industry and the consuming public.*	Madrid, Spain	Yes	5-A-1
UR-E29-(10)-37	Studies on the quantitative measurement of properties of wheat kernels that vary significantly during conditioning, as a basis for improved conditioning of wheat for milling by new and improved methods and increased industrial utilization of flour and milled wheat products.	St. Albans, England	Yes	2-C-5
UR-E29-(10)-39	A fundamental study of factors governing the onset of oxidative rancidity in oat products, to provide a basis for improving the quality and increasing the utilization of oats in feed and food.*	St. Albans, England	Yes	3-A-4

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PL 480 Research Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
UR-E29-(10)-40	Investigations of the structure and properties of cereal starches--particularly corn and wheat starches--as revealed by their interaction with enzymes and other proteins, to obtain fundamental information concerning the structure and behavior of cereal starches that would be useful in starch processing.*	Edgbaston, Birmingham, England	Yes	1-A-9
UR-E29-(10)-51	Investigation of sugars, their phosphate derivatives, and related compounds, as found in molds important to the fermentative conversion of cereal grains to useful products.	Newcastle-upon-Tyne, England	Yes	5-A-3
UR-E29-(10)-69	Fundamental studies on the nature and specificity of starch- and glycogen-debranching enzymes and the application of these enzymes to a study of the fine structures of amylopectins, amyloses, and glycogens of cereal grains, to provide a basis for increased utilization of cereal grains.	London, England	Yes	1-A-9
UR-E29-(10)-71	Investigations on the mechanism and structural changes involved in thermal, acid, or alkali degradation of cereal starches, to provide basic information for the development of new starch products suited for industrial applications.**	Edinburgh, Scotland	Yes	1-A-9
UR-E30-(10)-1	Studies on the modification of cereal grain starches by physical treatment of granular starch under different conditions of moisture, temperature, and pressure in order to impart new paste properties as a basis for increased utilization of cereal grains.	Ljubljana, Yugoslavia	Yes	1-B-6
UR-S3-(10)-11	Preparation of cationic cereal starch derivatives for use in paper and textiles by the introduction of quaternary phosphonium and tertiary sulfonium groups into crosslinked and noncrosslinked starches, to create new markets and expand old markets for starch from cereal grains.	Rio de Janeiro, Brazil	Yes	1-B-6
(10,40)	Cereal, forage crops, and oilseeds.			
UR-A11-(10,40)-10	Investigation of crosses of Saccharomyces rouxii isolated from the soybean fermentations, shoyu and miso, and an evaluation of their fermentative abilities in the above fermentation processes, as a basis for increasing the use of soybeans in fermented foods.	Noda-shi, Chiba-ken, Japan	Yes	8-B-3
(40)	Oilseeds			
UR-A6-(40)-1	Investigation of the various processes used in preparing Chinese cheese by the fermentation of soybean curd with Mucor and other fungi as a basis for increasing the foreign utilization of soybeans.	Taipei, Taiwan	Yes	8-B-5
UR-A7-(40)-21	Exploratory investigations of selected hydroxylated derivatives of linseed and safflower oils, to determine the feasibility of producing new industrial products from these oils.	Hyderabad, India	Yes	6-B-3

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PL 480 Research Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
UR-A7-(40)-95	Laboratory studies on the fermentative production of microbial lipases that are useful in converting vegetable oils to products of higher value as a basis for increasing the utilization of soybean and linseed oils.**	Baroda, India	No	
UR-A10-(40)-17	Fundamental investigations of complexes formed by soybean proteins with other meal constituents, to provide information for applied studies on expanded utilization of soybean oil meal.	Rehovot, Israel	Yes	8-A-3
UR-A10-(40)-18	Investigations of soybean saponins as related to the processing of petroleum ether-extracted meal for feed and to the preparation of soy foods, to provide information basic to improving the nutritional value of soybean protein products.	Rehovot, Israel	Yes	8-C-1
UR-A10-(40)-20	Laboratory investigations on miso-type food products by fermentation of soybean meal products and cereal grains for use in Israeli foods.	Ramat Gan, Israel	Yes	8-B-3
UR-A10-(40)-30	Investigations of the effect of processing conditions on the yield and quality of isolated soybean protein for use in Israeli-type foods, as a contribution to expanded utilization of soybeans.	Haifa, Israel	Yes	8-B-4
UR-A11-(40)-2	Evaluation of dehulled soybean grits from United States varieties for making miso, to increase soybean utilization in Japan.	Tokyo, Japan	Yes	8-B-3
UR-A11-(40)-5	Investigation of the partial hydrogenation of soybean oil, to produce a stable liquid oil with improved properties for use in Japanese foods.	Kawagoe, Saitama-ken, Japan	Yes	7-B-4
UR-A11-(40)-8	Isolation and determination of the flavor components of enzymatically or chemically modified soybean meal and proteins, and elucidation of their chemical and physical properties, to provide information basic to improving the flavor and thus increasing the utilization of soybeans.**	Tokyo, Japan	No	
UR-A11-(40)-11	Evaluation of United States soybean varieties as a material for producing fresh tofu to increase utilization in Japan.	Tokyo, Japan	No	
UR-A11-(40)-12	A chromatographic study of the sugars and oligosaccharides in soybeans to provide information needed to improve processing of fat-free soybean meal for foods and feeds, thereby contributing to its expanded utilization.	Takamatsu, Japan	Yes	8-A-2
UR-A11-(40)-13	Isolation, characterization, and quantitative determination of the sterols in soybeans to provide basic information for the evaluation and improvement of soybean meal and soybean products as foods and feeds.	Tokyo, Japan	Yes	8-A-4
UR-A11-(40)-14	Fundamental studies on color reversion of edible soybean oil to obtain information on its cause and prevention, as a means of increasing the utilization of soybean oil for food purposes in Japan.**	Tokyo, Japan	No	

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PL 480 Research Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

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UR-E8-(40)-2	Investigation of continuous multi-stage countercurrent crystallization of linseed and soybean fatty acids as a practical method for producing pure unsaturated fatty acids, to provide a basis for new or improved uses of linseed and soybean oils.*	Helsinki, Finland	Yes	7-C-3
UR-E15-(40)-8	An investigation of the minor constituents of linseed oil and their effect on the ability of linseed oil films to spread and adhere to surfaces, as a contribution to the expansion of markets for linseed oil.*	Milan, Italy	Yes	6-A-1
UR-E15-(40)-9	Investigations of the controlled thermal polymerization of soybean and linseed oils, and of the separation and characterization of the reaction products, in order to obtain information useful in expanding and improving the industrial applicability of these oils.*	Milan, Italy	Yes	6-B-2
UR-E15-(40)-10	Investigations of the effect of metallic catalysts and physical conditions on oxidative cleavage products produced in the autoxidation of polyunsaturated fatty acids, to provide basic information for applied research on the production of new industrial chemicals from soybean and linseed oils.	Milan, Italy	Yes	7-C-4
UR-E15-(40)-46	Studies on the effect of stereospecific polymerization catalysts on fatty esters from soybean and linseed oils, to provide a basis for increasing the industrial utilization of these vegetable oils.**	Milan, Italy	Yes	6-B-2
UR-E15-(40)-48	Synthesis and use of lipid-soluble metal chelates of Schiff bases as catalysts for the selective hydrogenation of soybean oil, to provide a basis for improving its flavor stability for edible use.**	Milan, Italy	No	
UR-E21-(40)-6	Chromatographic determination of the glyceride composition of selected erucic-acid containing oils, to provide basic information important to their utilization.	Warsaw, Poland	Yes	9-A-2
UR-E21-(40)-8	Investigation of the possible role of sterols in the development of flavors and odors in soybean oil through studies of sterol transformations during processing, in order to increase the utilization of soybeans in food.	Gdansk, Poland	Yes	7-A-4
UR-E25-(40)-4	Investigations of ion exchange procedures for removing pro-oxidant metals from soybean oil, in order to contribute to expanded utilization of soybean oil through improvement of its flavor and oxidative stability during transportation, storage, and use.*	Seville, Spain	Yes	7-A-3
UR-E25-(40)-29	Improvement of the frying quality of soybean oil through studies of the influence of processing factors and oil modifications on surface tension, interfacial tension, viscosity, and other physical properties concerned with its penetration into fried foods, to provide information for increased use in the preparation of Spanish foods.	Granada, Spain	Yes	7-B-3

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PL 480 Research Project Check List -- Reporting Year July 1, 1964 to June 30, 1965 (Cont'd.)

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UR-E26-(40)-3	Compositional investigations of Swedish Cruciferae (mustard family) seeds to find strains with maximum erucic acid content in their oils and minimum content of glucosidic precursors of isothiocyanates and thiooxazolidones in their meals, to provide a basis for their utilization as industrial oilseeds in the United States.	Svalof, Sweden	Yes	9-A-1
UR-E29-(40)-29	Development of new uses for soybean and linseed oils through investigations of organometallic derivatives and complexes as components of protective coatings having improved properties.*	Teddington, Middlesex, England	Yes	6-C-4
UR-E29-(40)-49	Investigation of the reactions of unsaturated fatty acids and their derivatives in molten alkali, to discover new chemical intermediates important to the increased utilization of soybean and linseed oils.	London, England	Yes	7-C-4
UR-E29-(40)-50	A quantitative study of the polysaccharides in fat-free soybean meal to provide information needed to improve the processing of meal for foods and feeds, thereby contributing to its expanded utilization.	Edinburgh, Scotland	Yes	8-A-2
(50)	Sugar and miscellaneous crops.			
UR-E15-(50)-29 (Rev.)	Preparation and characterization of dextran derivatives, and investigations of their interactions and binding, to provide basic information for increasing the utilization of sugar.	Rome, Italy	Yes	10-A-2

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